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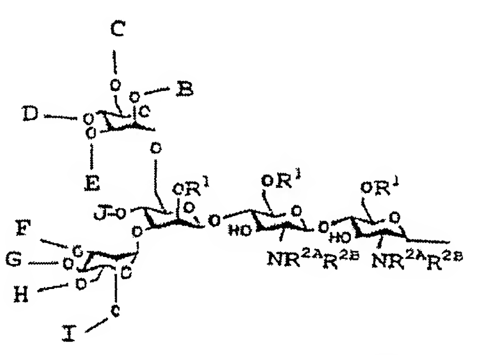
(54) Title: GLYCOPEPTIDE DIMERS AND USES THEREOF



(I)

(57) Abstract: This invention provides compounds represented by the formula: $(A\text{-}X\text{---})_n$wherein each A- is independently a carbohydrate represented by the general structure: wherein each of B, C, D, E, F, G, H, I, and J independently represents a sugar moiety, a sugar moiety having a protecting group attached thereto, a hydrogen, or an oxygen protecting group, provided that no more than two of B, C, D, and E is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and that no more than one of F, G, H, and I is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and wherein represents a covalent bond between the carbohydrate and the peptide; wherein each R^1 independently represents a hydrogen or an oxygen protecting group; wherein each R^{2A} and each R^{2B} is independently a hydrogen or a nitrogen protecting group; wherein X is a peptide; wherein --- represents a disulfide bond; and wherein n represents an integer ≥ 2.

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GLYCOPEPTIDE DIMERS AND USES THEREOF

This invention was made with support under United States Government Grant No. CA-28824 from the National Institute of Health, U.S. Department of Health and Human Services. Accordingly, the United States Government has certain rights in the invention.

This application claims the benefit of U.S. Provisional Application No. 60/586,859, filed July 9, 2004, the contents of which are hereby incorporated by reference into this application.

Throughout this application, various publications are referenced. Full citations for these publications are found at the end of the specification immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art known to those skilled therein as of the date of this invention.

Background of the Invention

Until very recently, extensive glycosylation of HIV envelope proteins had been considered to be one of the major impediments to the development of an HIV vaccine. Indeed, this "glycan shield" was perceived to confer protection from antibodies which would recognize the peptide backbone of the gp120 trimer surface. The envelope glycoprotein gp120 interacts sequentially with the cellular receptor CD4 and a member of the chemokine receptor family, thus initiating HIV entry into the T-cell. The gp120 peptide chain is heavily

glycosylated, typically bearing 24 Asn-linked glycans. The idea of utilizing gp120 carbohydrates as antigens for eliciting broadly neutralizing immune responses gained recognition only when the structure of the 2G12 antibody epitope was unveiled. This antibody, isolated from a long-term survivor of infection, was shown to efficiently neutralize a wide spectrum of different HIV isolates in vitro and to protect macaques from simian-human immunodeficiency virus challenge.

The human mAb 2G12 is one of the few monoclonal antibodies capable of neutralizing a broad range of primary HIV-1 isolates.

Recent studies have emphasized the role of N-linked glycans in gp120 as being critical for recognition by 2G12. In brief, the studies listed below can be summarized as follows: a cluster of N-linked glycans at positions N295, N332, and N392 within gp120 are most critical for binding the 2G12 antibody.

Sanders et al. (*J. Virol.* Vol. 76: pp. 7293-7305, 2002) digested recombinant gp120 with various glycosidase enzymes of known specificities and showed that the 2G12 epitope is lost when gp120 is treated with mannosidases. Sanders et al. also used computational analyses to position the epitope in the context of the virion-associated envelope glycoprotein complex. Together, the analyses suggested that the 2G12 epitope is centered on the high-mannose and/or hybrid glycans of residues N295, N332, and N392, with peripheral glycans from N386 and N448 on either flank. Sanders et al. then concluded that the epitope is mannose-dependent and composed primarily of carbohydrate, with probably no direct involvement of the gp120 polypeptide surface. The epitope resides on the face

orthogonal to CD4 binding face, on a surface proximal to, but distinct from, the surface implicated in co-receptor binding.

Similar conclusions as to the role of gp120 glycosylation for 2G12 binding were reached in a study by Scanlan et al. (*J. Virol.* Vol. 76: pp. 7306-7321, 2002). The 2G12 epitope of gp120 was investigated using site-directed mutagenesis and carbohydrate analysis. Using extensive alanine-scanning mutagenesis, it was established that elimination of the N-linked carbohydrate attachment sequences associated with residues N295, N332, N339, N386, and N392 produced significant decreases in 2G12 binding affinity to gp120. They further showed that the glycans at N339 and N386 were not critical for binding to gp120, consistent with sequence comparisons among isolates neutralized by 2G12. They obtained no convincing evidence for the involvement of gp120 amino acid side chains in 2G12 binding. Antibody binding was inhibited when gp120 was treated with Aspergillus saitoi mannosidase, Jack Bean mannosidase, or endonuclease H, indicating Manα1-2Man-linked sugars of oligomannose glycans on gp120 are required for 2G12 binding. Consistent with this finding, the binding of 2G12 to gp120 could be inhibited by monomeric mannose, but not by galactose, glucose, or N-acetylglucosamine. The inability of 2G12 to bind to gp120 in the presence of the glucose analogue N-butyl-dioxynojirimycin similarly implicated Manα1-2Man-linked sugars in 2G12 binding. The authors conclude that the most likely epitope for 2G12 is formed from mannose residues contributed by the glycans attached to N295 and N332 with the other glycans playing an indirect role in maintaining epitope conformation.

Calarese et al. (*Science* Vol. 300: pp. 2065-2071, 2003) investigated the crystal structures of Fab 2G12 in complexes

with the disaccharide Manα1-2Man and with the high-mannose oligosaccharide Man(9)GlcANAc(2) and found that two Fabs assemble into an interlocked VH domain-swapped dimer. Biochemical, biophysical, and mutagenesis data strongly support a Fab-dimerized antibody as the prevalent form that recognizes gp120. The extraordinary configuration of this antibody provides an extended surface, with newly described binding sites, for interaction with a conserved cluster of oligomannose type sugars on the surface of gp120.

Li et al. (The Royal Society of Chemistry 2004, pages 483-488) chose cholic acid as the scaffold for three high-mannose type oligosaccharides to design more effective epitope mimics for antibody 2G12.

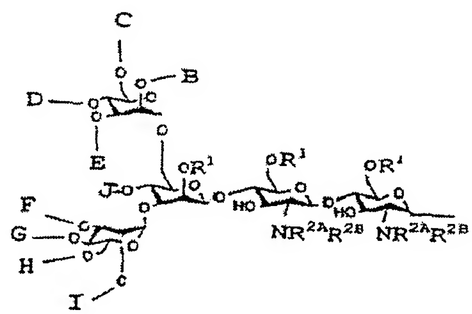
The present invention provides compounds in which high-level binding is an exclusive property of multimeric glycopeptides. Such better binding may not be limited to dimers, and may also be a property of trimers, tetramers, or higher-order species. The present invention provides advantages over the prior art, namely, flexibility in valency, flexibility in distance between glycans, ability to use the native gp120 peptide backbone, and ease of incorporation of the peptide sequences that bind MHC and stimulate T-cell help.

Summary of the Invention

This invention provides compounds represented by the formula:

$$(A\sim X---)_n$$

wherein each A~ is independently a carbohydrate represented by the general structure:



wherein each of B, C, D, E, F, G, H, I, and J independently represents a sugar moiety, a sugar moiety having a protecting group attached thereto, a hydrogen, or an oxygen protecting group, provided that no more than two of B, C, D, and E is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and that no more than one of F, G, H, and I is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and wherein ~ represents a covalent bond between the carbohydrate and the peptide; wherein each R^1 independently represents a hydrogen or an oxygen protecting group; wherein each R^{2A} and each R^{2B} independently represents a hydrogen or a nitrogen protecting group; wherein X is a peptide comprising 5-20 amino acids, at least one amino acid being a cysteine residue and at least one other amino acid are being an asparagine or a glutamine residue; wherein --- represents a disulfide bond between the sulfur of a cysteine in one peptide and the sulfur of another cysteine in another peptide; and wherein n represents an integer ≥ 2.

This invention also provides a composition comprising the above compound and a carrier.

This invention further provides a method of eliciting an immune response against HIV-1 or an HIV-1 infected cell in a subject which comprises administering to the subject an amount of the above compounds or a dose of the above compositions in effective to elicit the immune response.

Brief Description of the Figures

Figure 1a-1b

(a) Analyses of binding to 2G12 (Signals for **17** and **19** overlap at the baseline)

(b) Analysis of DTT effect on binding

Figure 2.

Competition binding data for glycopeptide **15** and gp120. Sensograms on the right top and bottom are normalized before injections of gp120 and compound **15**, respectively.

Figure 3.

Glycopeptide **15** binding profiles at 1.25, 2.5, 5, 10 μM concentration.

Figure 4.

Binding assay for gp120 and the gp120 glycopeptide specifically to 2G12.

Figures 5a-5d.

Binding assays for selected glycans

Figure 6.

Comparison of binding profiles for several compounds suggests that high-mannose type glycan attached to the peptides is essential for binding to 2G12

Figures 7a-7b.

(a) Compound A shows reduced binding to a 2G12 surface if this surface has been pre-blocked by gp120.

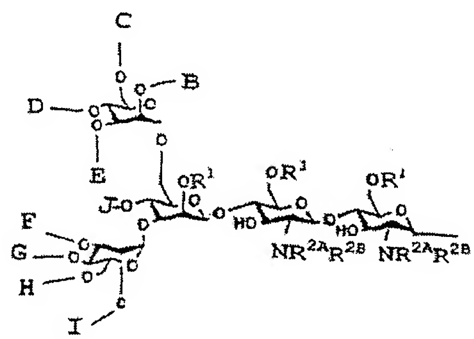
(b) In a reciprocal experiment, gp120 appears to show reduced binding to a 2G12 surface pre-blocked by compound A.

Detailed Description of the Invention

This invention provides compounds represented by the formula:

$$(A\sim X---)_n$$

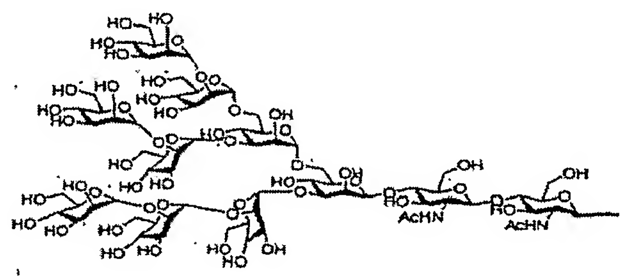
wherein each A~ is independently a carbohydrate represented by the general structure:



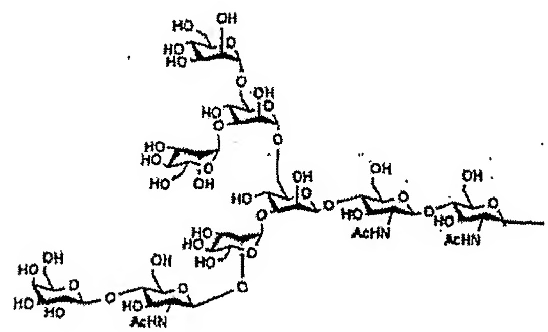
wherein each of B, C, D, E, F, G, H, I, and J independently represents a sugar moiety, a sugar moiety having a protecting group attached thereto, a hydrogen, or an oxygen protecting group, provided that no more than two of B, C, D, and E is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and that no more than one of F, G, H, and I is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and wherein ~ represents a covalent bond between the carbohydrate and the peptide; wherein each R^1 independently represents a hydrogen or an oxygen protecting group; wherein each R^{2A} and each R^{2B} independently represents a hydrogen or a nitrogen protecting group; wherein X is a peptide comprising 5-20 amino acids, at least one amino acid being a cysteine residue and at least one other amino acid are being an asparagine or a glutamine residue; wherein --- represents a disulfide bond between the sulfur of a cysteine in one peptide and the sulfur of another cysteine in another peptide; and wherein n represents an integer ≥ 2.

This covalent bond may be such that the carbohydrate is beta-bound or alpha-bound to the peptide.

In one embodiment, this invention provides such compounds, wherein each A is independently a carbohydrate having one of the following structures:



; or

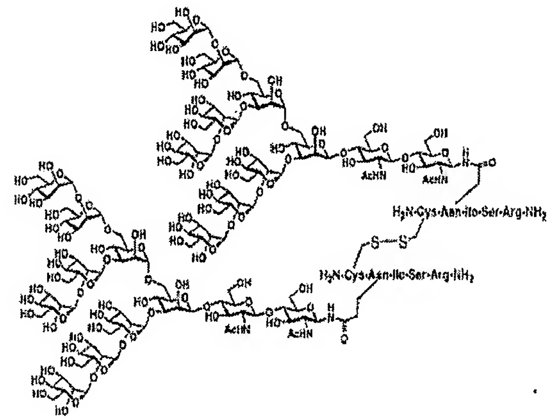


In a preferred embodiment, the peptide comprises consecutive amino acids, the sequence of which is present in gp120 of HIV virus.

In general in the compounds of the invention, ~ represents a covalent bond between a carbohydrate and an amide moiety of an asparagine or a glutamine residue present on the peptide.

In certain embodiments of the invention, n equals 2.

In one embodiment, the compound of the invention has the following structure:



In another embodiment, the invention provides compositions comprising one or more of the above described compounds and a carrier, e.g. a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

In general the above compositions will further comprise an immune response stimulating amount of an immunostimulatory adjuvant, e.g. QS21, BCG or the like. Preferably, the compound or compounds are present in the composition in an amount effective to elicit a prophylactic and/or therapeutic response against HIV-1 or an HIV-1 infected cell.

This invention further provides a method of eliciting an immune response against HIV-1 or an HIV-1 infected cell in a subject which comprises administering to the subject an amount of any of the above described compounds or a dose of any of the above described compositions effective to elicit such an immune response and thereby protect against and/or treat an HIV-1 infection.

<u>Definitions</u>

Examples of compounds of the present invention, and definitions of specific functional groups are described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75^{th} Ed., inside cover, and specific functional groups are defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito (1999).

As used herein, "adjuvant" or "immunostimulatory adjuvant" means a moiety, which, when co-administered with an immunogen, enhances the immunogenicity of the immunogen. Adjuvants enhance the immunogenicity of an immunogen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the immunogen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of immunogen to cells of the immune system. Adjuvants can also attract cells of the immune system to an immunogen depot and stimulate such cells to elicit immune responses. As such, this invention encompasses compositions further comprising adjuvants. Adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants (such as lipopolysaccharides) normally are the components of killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause

undesirable side-effects making them unsuitable for use in humans and many animals. Indeed, aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established. Notwithstanding, it does have limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response with other immunogens. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents. In addition to adjuvants used for therapeutic purposes (e.g., vaccines), other adjuvants may be used for raising antibodies in animals, which antibodies may be used, for example, in diagnostic and immunoassays. Examples of such adjuvants include, but are not limited to, bacteria or liposomes. For example, suitable adjuvants include but are not limited to, saponin adjuvants (*e.g.*, GPI-0100), *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21. A wide range of extrinsic adjuvants can provoke potent immune responses to immunogens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

As used herein, "amino acid" refers to any one of the common, naturally occurring L-amino acids found in naturally occurring proteins: glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), arginine (Arg), histidine (His), proline (Pro), serine (Ser), threonine (Thr),

phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), cysteine (Cys) and methionine (Met); or any of the unnatural amino acid, or D-amino acids.

As used herein, "eliciting an immune response" means initiating, triggering, causing, enhancing, improving or augmenting a response of the immune system, for example, of either a humoral or cellular nature. The initiation or enhancement of an immune response can be assessed using assays known to those skilled in the art including, but not limited to, antibody assays (for example ELISA assays). In certain exemplary embodiments, the inventive gp120 glycans and/or glycoconjugates thereof, and the methods of the present invention essentially trigger or enhance primarily a humoral immune response.

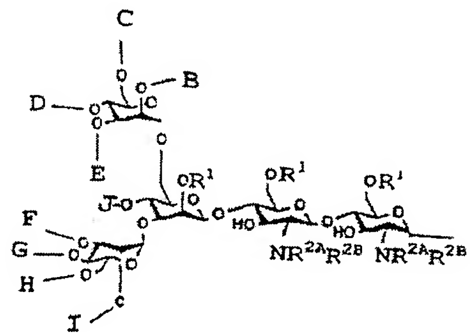
As used herein, "protecting group" means that a particular functional moiety, *e.g.*, O or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents, that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. For example, certain oxygen protecting groups may be utilized. These oxygen protecting groups include, but are not limited to methyl ethers, substituted methyl ethers (*e.g.*,

MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM or MPM (p-methoxybenzyloxymethyl ether), to name a few), substituted ethyl ethers, substituted benzyl ethers, silyl ethers (*e.g.*, TMS (trimethylsilyl ether), TES (triethylsilylether), TIPS (triisopropylsilyl ether), TBDMS (t-butyldimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyldiphenyl silyl ether), to name a few), esters (*e.g.*, formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. Nitrogen protecting groups may also be utilized. These nitrogen protecting groups include, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (*e.g.*, Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. In addition, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

Compounds of this invention are also represented by the formula:

$$(A_n\sim Y)$$

wherein each A is independently a carbohydrate represented by the general structure:



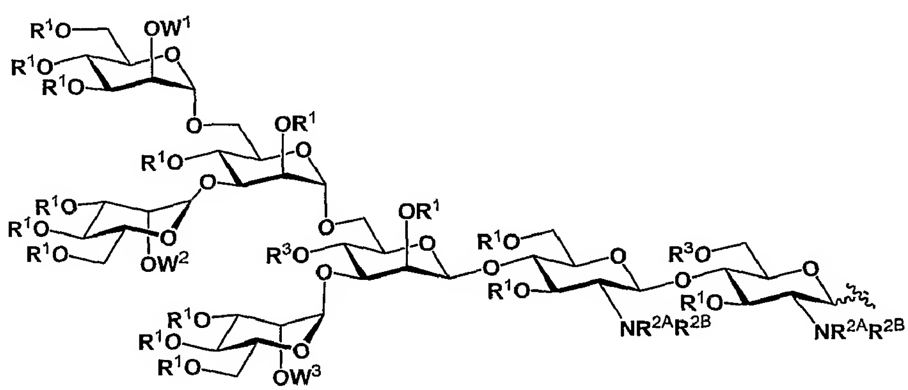
wherein each of B, C, D, E, F, G, H, I, and J independently represents a sugar moiety, a sugar moiety having a protecting group attached thereto, a hydrogen, or an oxygen protecting group, provided that no more than two of B, C, D, and E is simultaneously a sugar moiety or a sugar moieties having a protecting group attached thereto and that no more than one of F, G, H, and I is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and wherein ~ represents a covalent bond between the carbohydrate and the peptide; wherein each R^1 independently represents a hydrogen or an oxygen protecting group; wherein each R^{2A} and each R^{2B} is independently a hydrogen or a nitrogen protecting group; wherein Y is a compound comprising multiple carboxyl groups prior to reaction with A; and wherein n represents an integer ≥ 2.

This invention further provides the above compounds, wherein Y is a peptide containing multiple aspartate and/or glutamate groups. The peptide may be derived from, or contain an identical sequence to one within, HIV-1 gp120 and/or comprise epitopes which are known to promote binding to major histocompatibility (MHC) molecules, either class I or class II MHC molecules. The peptides may be longer than 20 amino acids in order to incorporate multiple glycans and MHC epitopes.

The determinants of peptide binding to MHC molecules are well-known in the art. (See, for example, Sung M.H., et al., *J. Comput. Biol.* (2004) 11(1):125-145; Surman S., et al., *Proc. Natl. Acad. Sci. USA* (2001) 98(8):4587-4592; and Ahlers J.D., et al., *Proc. Natl. Acad. Sci. USA* (1997) 94(20):10856-10861). Alternatively, this invention provides the above compound, wherein Y is a non-peptidic molecule.

In certain embodiments of the present invention, when Y is a peptide, the distances and spatial orientation between the multiple carbohydrates can be varied by changing the number and/or identity of the amino acids separating the multiple carboxyl-group-containing amino acids. As an example, praline can promote beta-hairpin conformations in peptides and proteins. Beta-pleated sheet structures can be stabilized via nonconvalent cross-strand interactions, such as hydrogen bonds, salt bridges, and von der Waals forces.

In certain embodiments of the present invention, A has the structure:



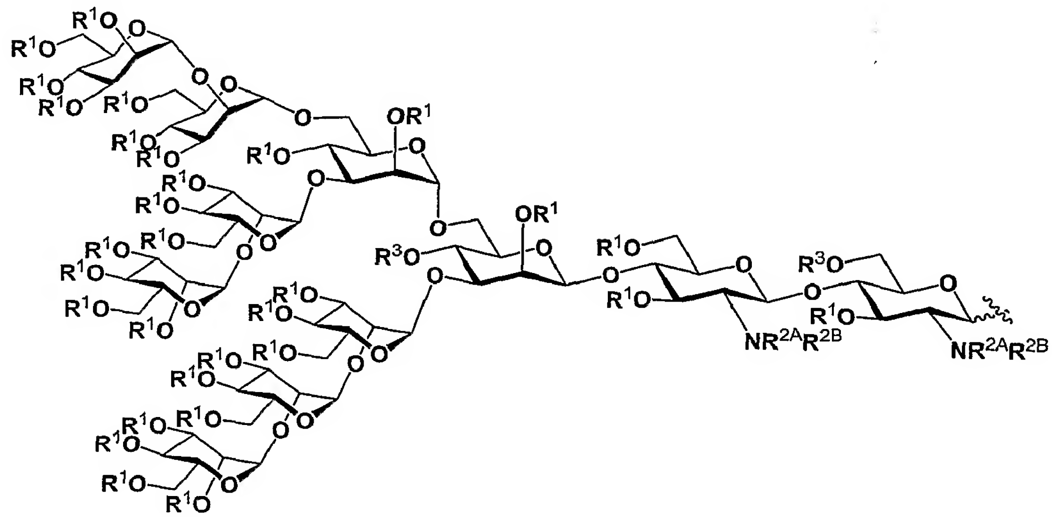
(I)

wherein each occurrence of R^1 and R^3 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

W^1, W^2 and W^3 are each independently optionally substituted mannose, galactose or lactosamine moieties;

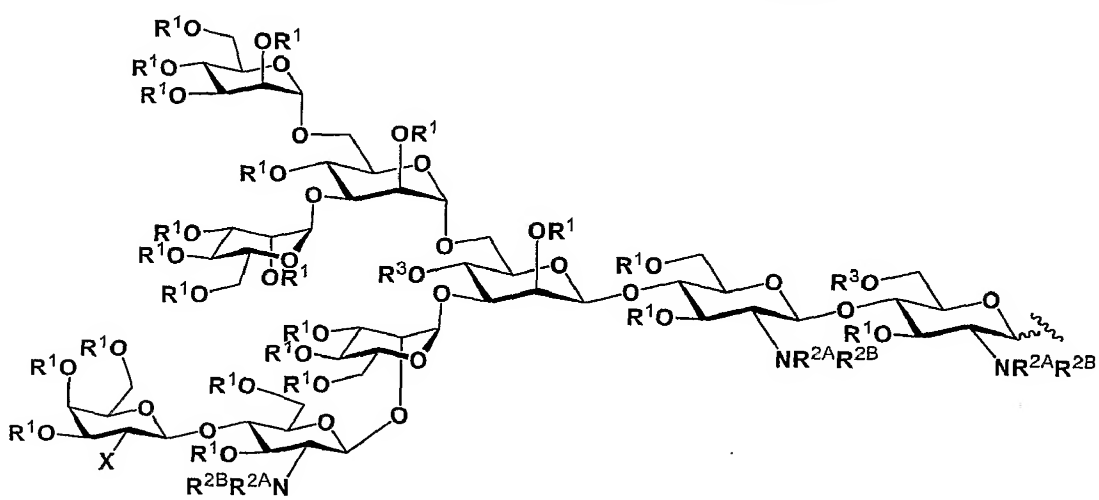
In other embodiments, A has the structure:



(II)

wherein R^1, R^3, R^{2A} and R^{2B} are each as defined above for **(I)**.

In yet other embodiments, A has the structure:

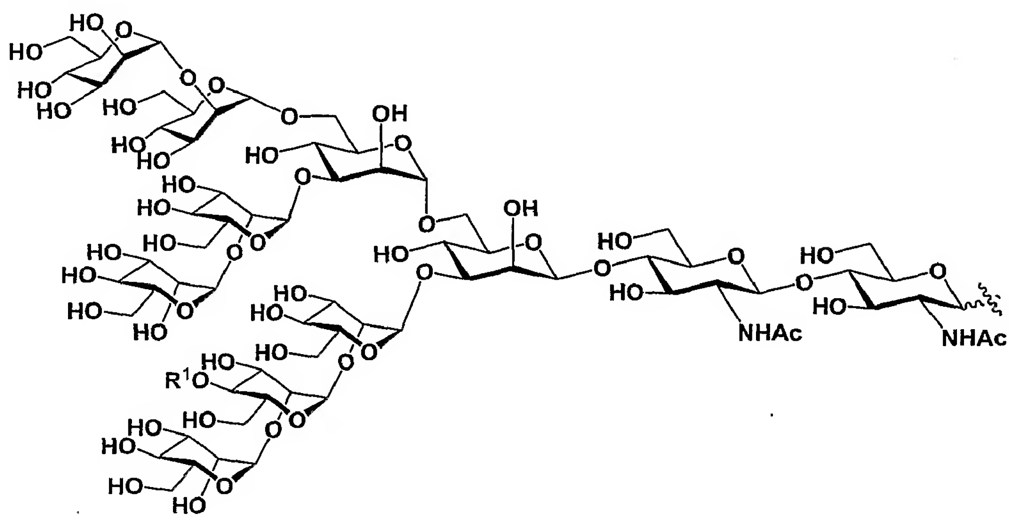


(III)

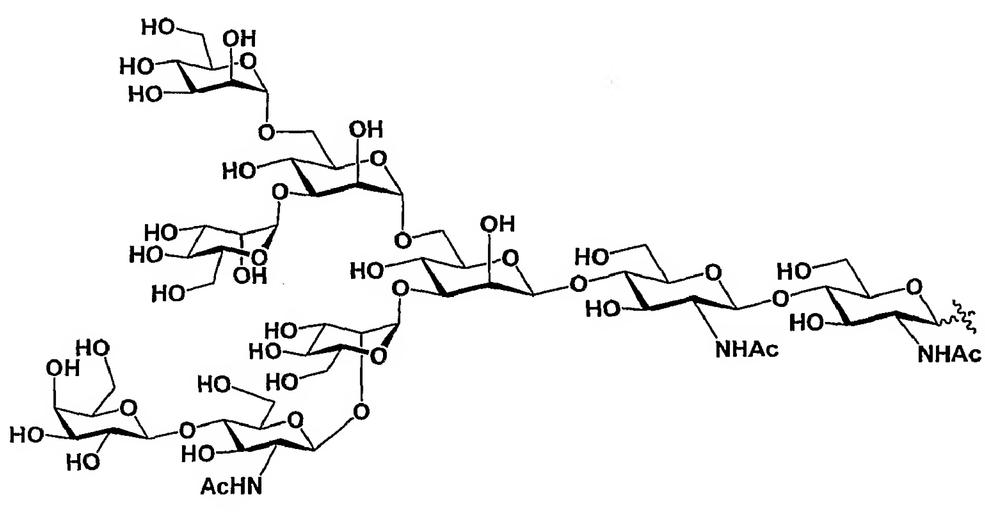
wherein R^1, R^3, R^{2A} and R^{2B} are as defined above for **(I)**.

Guidance for preparing certain embodiments of the invention comprising one or more carbohydrate domains of the formula **(I)**, **(II)** or **(III)** can be found, *inter alia*, in PCT International Application No. PCT/US03/038471 filed on December 3, 2003, the contents of which are hereby incorporated by reference.

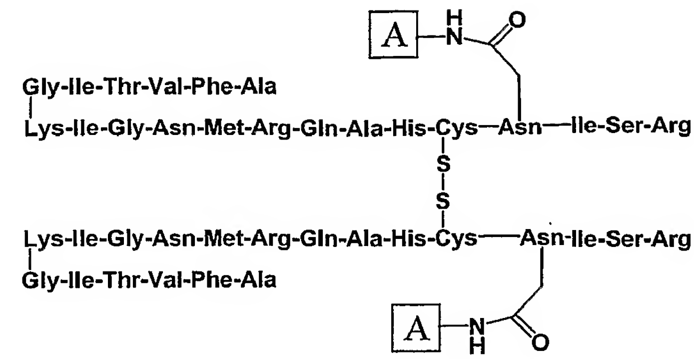
In certain embodiments, each occurrence of A is independently a carbohydrate domain having one of the following structures:



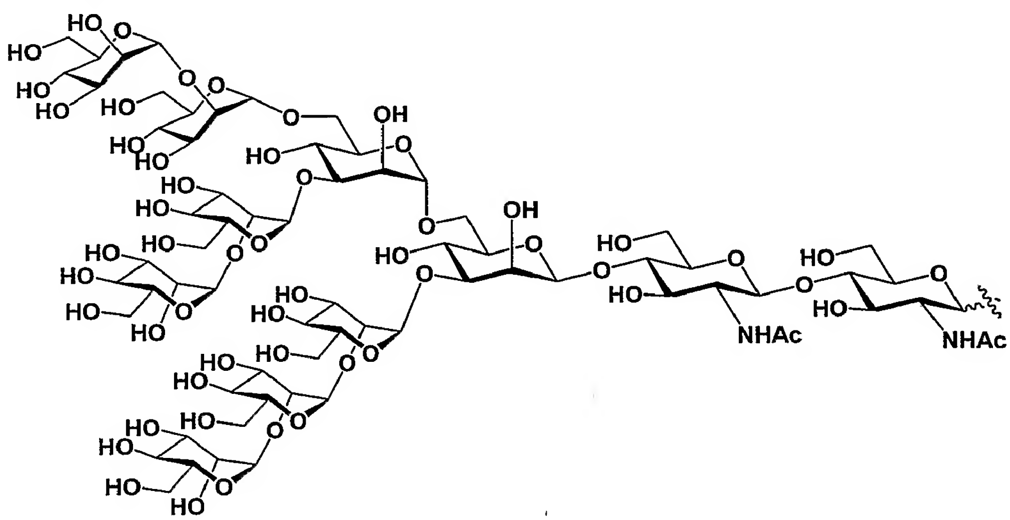
or



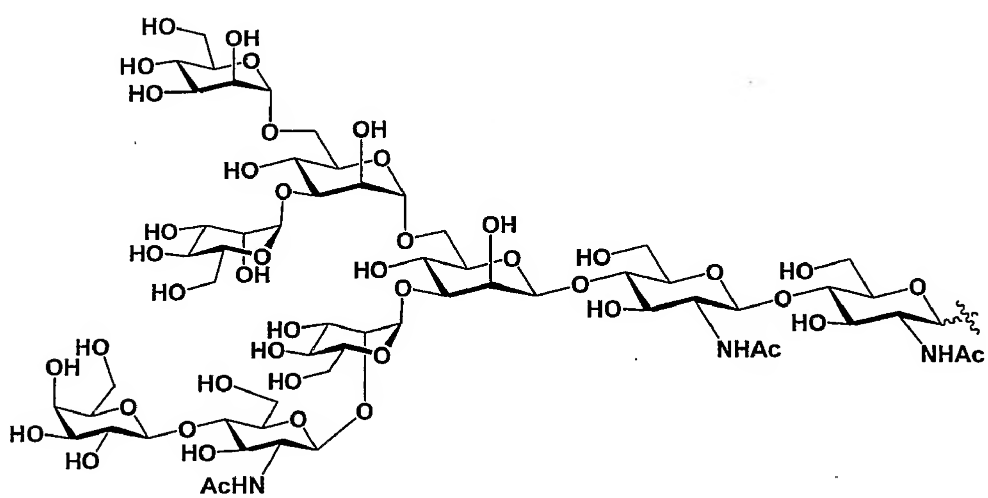
In certain embodiments, the invention provides dimeric compounds having the structure:



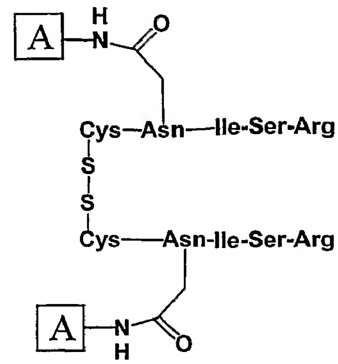
wherein each occurrence of A is independently a carbohydrate having one of the structures:



or



In certain embodiments, dimeric compounds having the following structure are provided:



wherein A is as defined above.

Experimental Details (Part I)

Synthetic Methodology

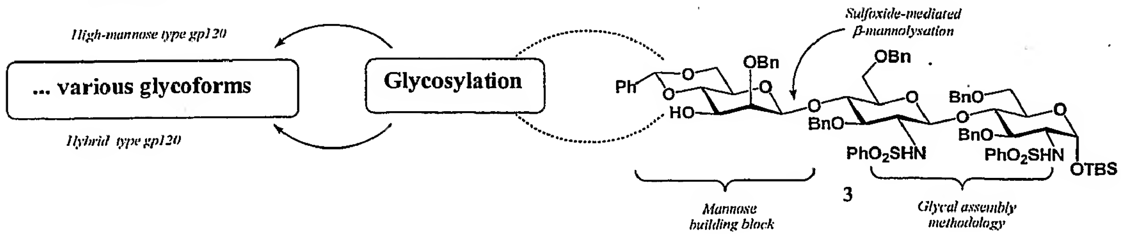
A well-established literature of carbohydrate chemistry exists and provides guidance, in combination with the information contained herein, on synthetic strategies, protecting groups, and other materials and methods useful for the synthesis of the compounds of this invention.

The various patents and other documents cited herein provide helpful background information on preparing compounds similar to the compounds described herein as well as relevant intermediates, information on formulation, uses, and administration of such compounds.

Glycan Synthesis

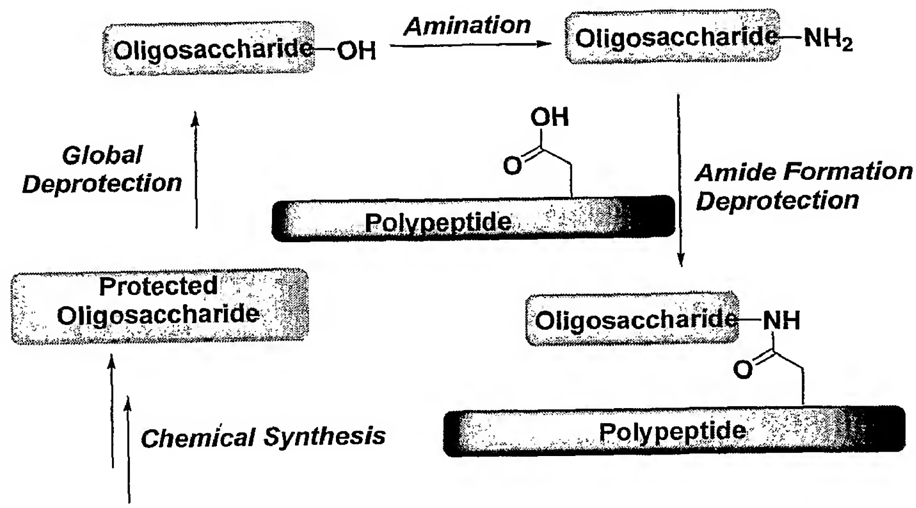
The following methods allowing access to a number of gp120-derived saccharides using only a small set of building blocks and the same general procedure for each glycan may be found in the aforementioned PCT International Application PCT/US03/038471.

Scheme 1. Proposed methodology for glycan synthesis.



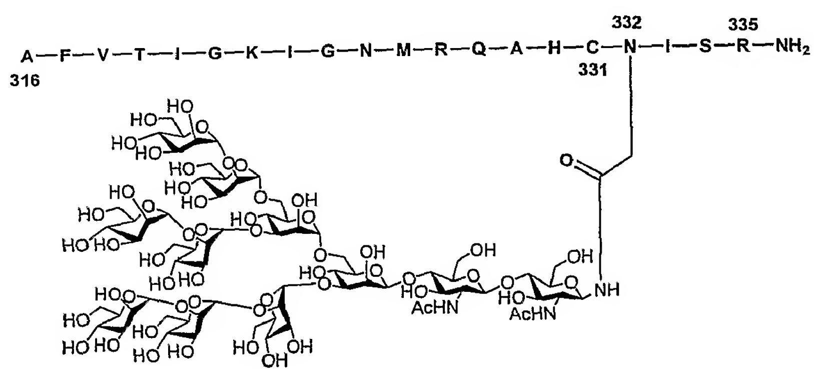
In one example, trisaccharide **3** in Scheme 1 embodies the protected core structure reported for the glycoforms expressed in gp120. In another example, trisaccharide **3** may be elaborated to give a pentasaccharide either by deprotection of the 6-position followed by simultaneous α-mannosylation at the free 3- and 6-positions or by sequential mannosylation at the 3-and 6-positions with an intermediate deprotection step. Simultaneous mannosylation with equivalently protected mannosyl donors would yield a "symmetrically" substituted pentasaccharide; further deprotections and glycosylations could be achieved in a synchronous fashion at both nonreducing termini. Sequential mannosylation would allow the inclusion of differentially protected mannose building blocks, permitting independent elaboration of the 3- and 6-substituted antennae. Thus the high-mannose pentasaccharide core (which is conserved in most natural *N*-linked glycans) may be synthesized in large quantities and used as a starting point for all of the gp120 targets. Moreover, because hybrid-type gp120 differs from high-mannose type gp120 in its degree of branching beyond the core pentasaccharide, this synthetic scheme would provide easy access to the multi-antennary glycoforms expressed in gp120.

Scheme 2. Exemplary synthetic strategy



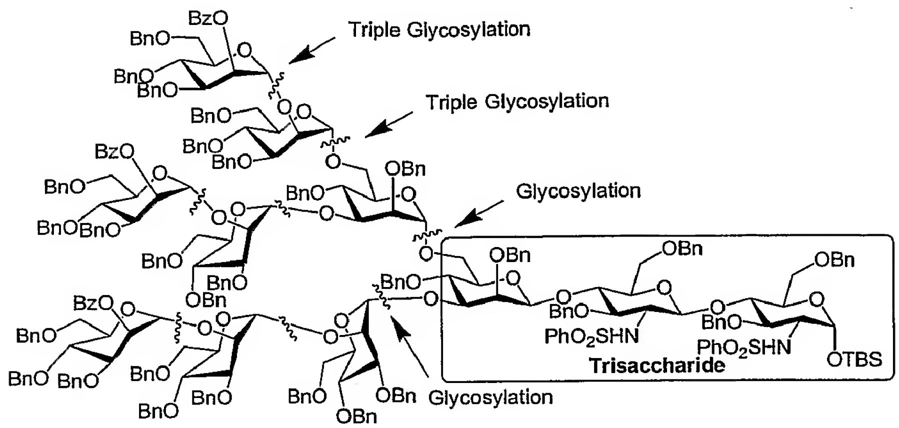
This synthetic approach includes: synthesis of protected oligasaccaride (undecassaccharide), global deprotection to prepare free glycan, amination, coupling with peptide acid and deprotection.

In one example, a synthesis for the high-mannose type glycopeptide having the structure:



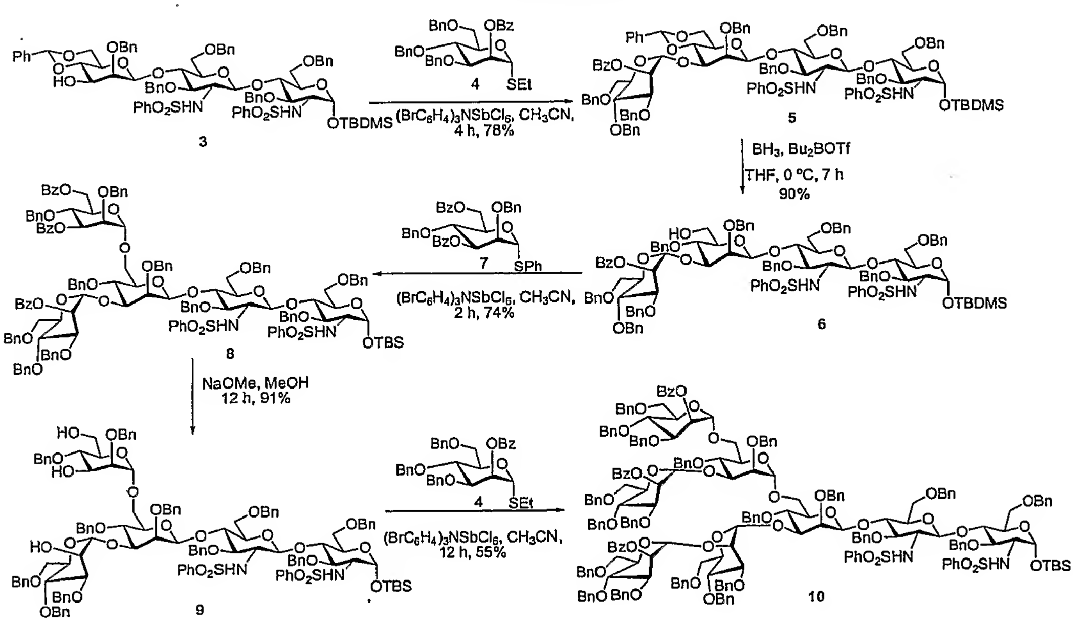
is provided.

Scheme 3. Exemplary retrosynthesis of undesaccharide **1.**



A synthetic plan for the preparation of the undecasaccharide is shown in Scheme 3. For example, starting from a trisaccharide intermediate (e.g., trisacchraide **3**),[1] two successive glycosylations will give pentasaccharide, then two consecutive triple glycosylation would furnish the undecasaccharide.

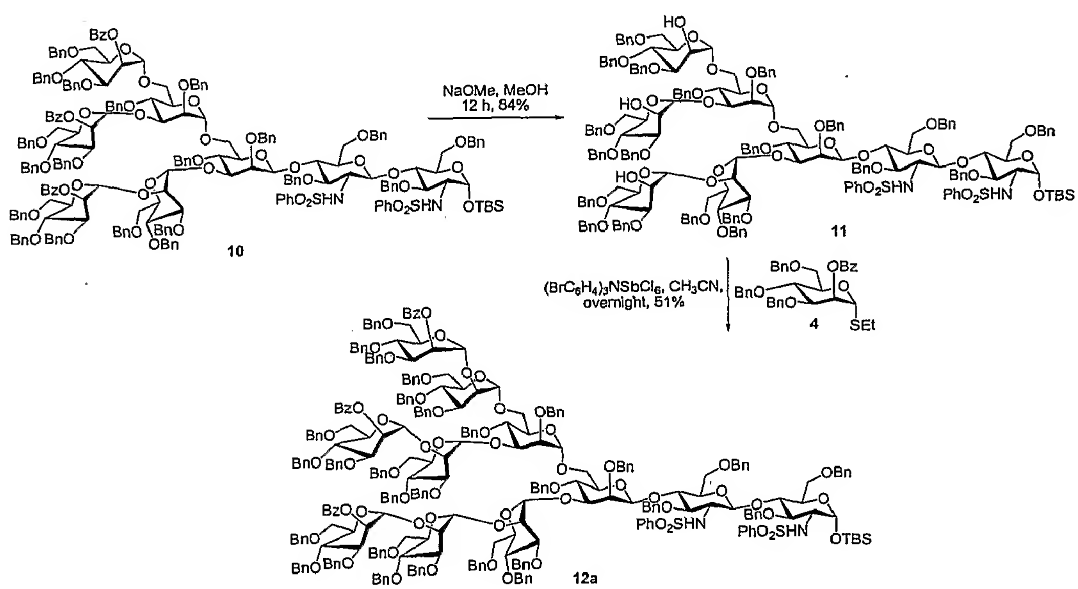
Scheme 4



An exemplary synthesis using this route is shown in Scheme 4. For example, mannosylation of trisaccharide **3** using

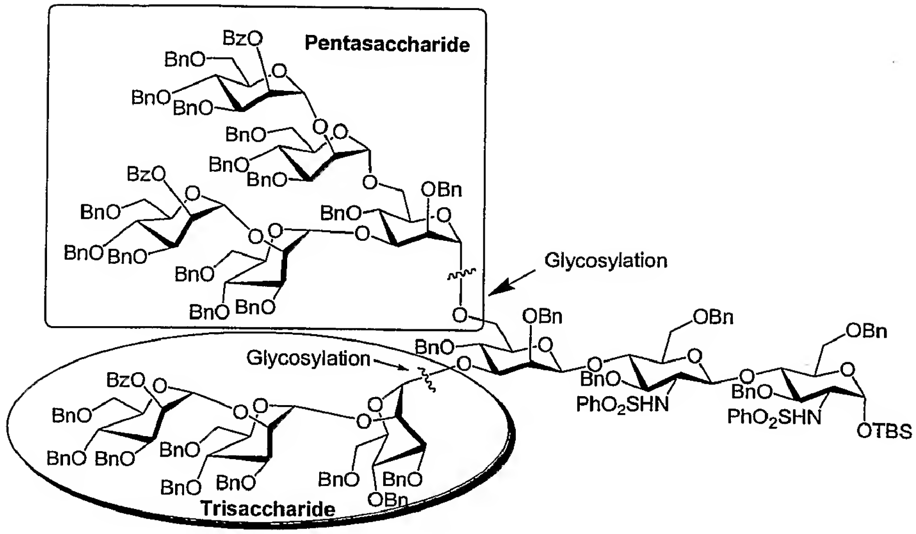
thiomannoside **4** and Sinaÿ radical cation activation[2,3] gave tetresaccharide in 78% yield. The benzylidene ring was reductively opened by borane and the resulting free alcohol **5** underwent mannosylation to give pentasaccharide **8** in 74% yield. After Zemplen reaction, the newly generated three free OH were mannosylated to afford octasaccharide **10** using same Sinaÿ conditions[2,3]. The same triple-glycosylation sequence was repeated to synthesize the undecsaccharide **12a** in 55% yield (Scheme 5)

Scheme 5



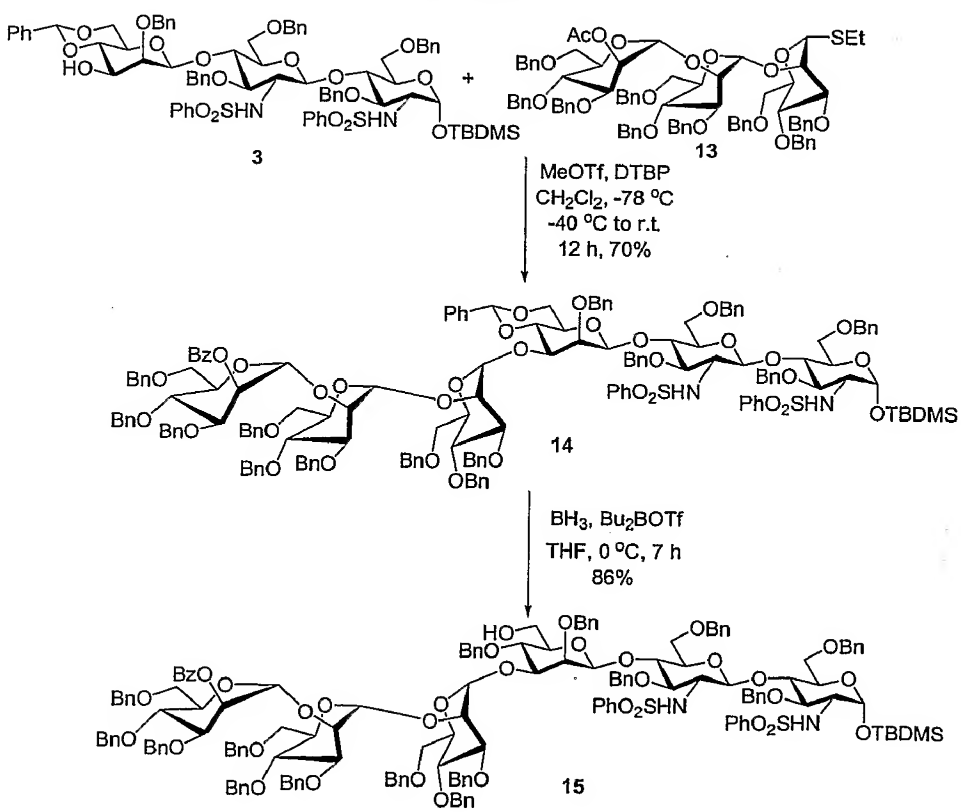
For example, as shown in Scheme 6, the desired undecasaccharide could be synthesized by a 3+3 glycosylation (trisaccharide couples with another trisaccharide) followed by a 6+5 coupling. This synthetic plan is much shorter and more convergent than the first strategy.

Scheme 6



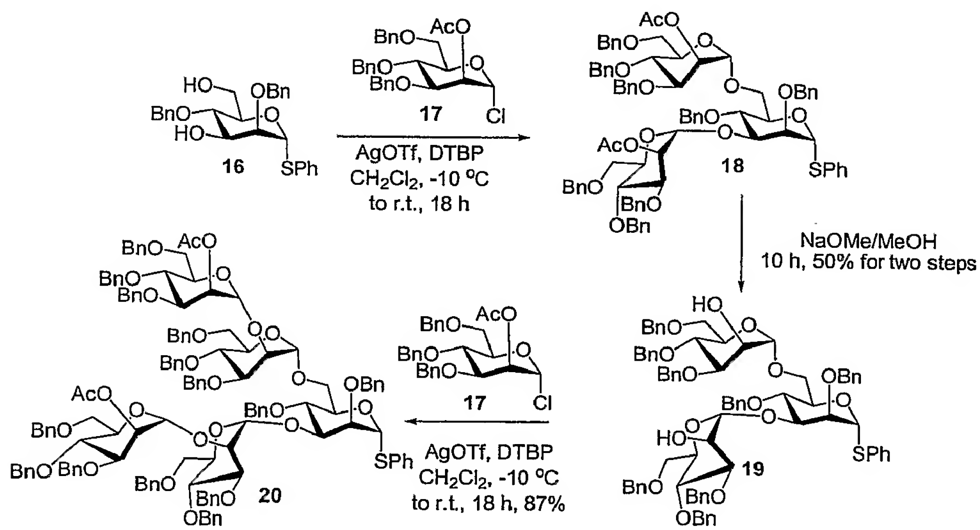
In one example, trisaccharide **3** first undergoes glycosylation with trisaccharide donor **13** using MeOTf as promoter to afford hexasaccharide in 70% yield. Then reductive ring-opening of the benzylidene ring gave saccharide **15** in 87% yield.

Scheme 7



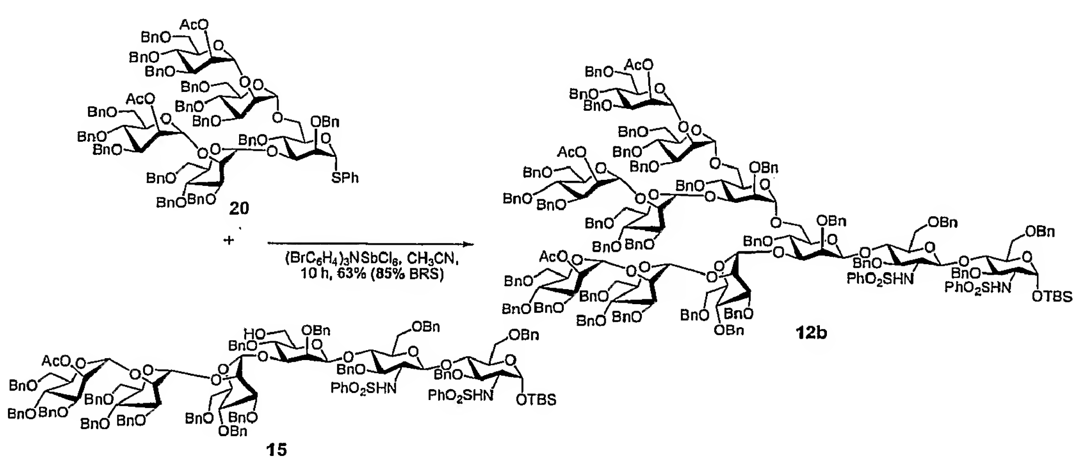
In another example, the pentasaccharide which is the precursor for the upper-left portion of the final compound **(1)** was synthesized as shown in scheme 8. Double-glycosylation of mannose derivative **16** using chloro donor **17** and promoter silver triflate gave trisaccharide **18**. After cleavage of the two acetyl groups, another double-glycosylation provided pentasaccharide **20** in 87% yield.

Scheme 8



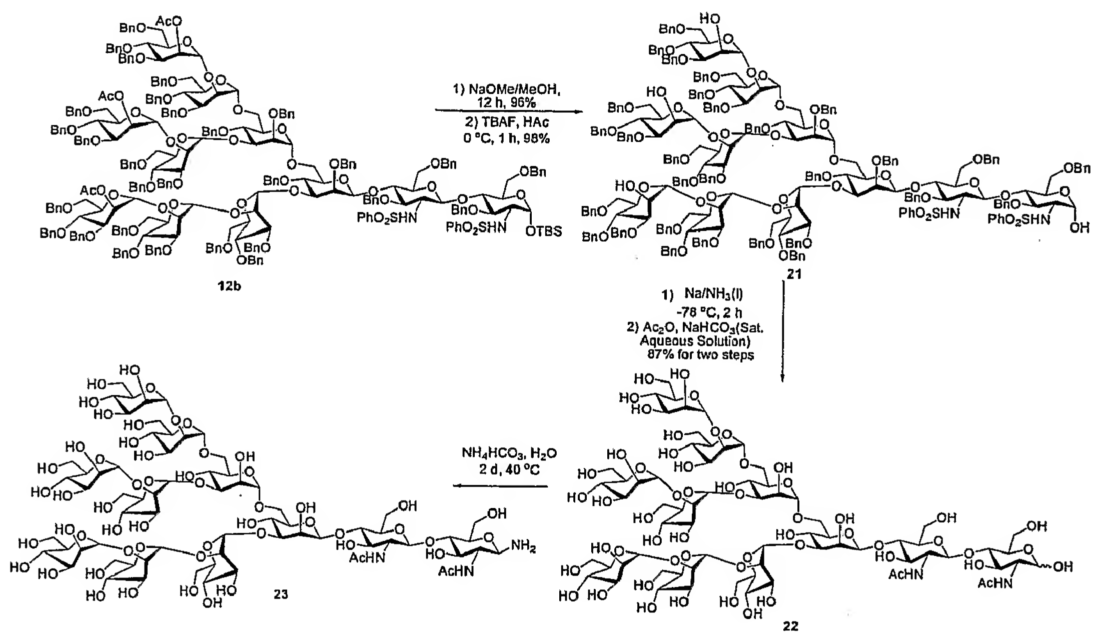
In certain embodiments, the 6+5 glycosylation using Sinaÿ radical cation activation[2,3] proceeds smoothly giving the desired undecasaccharide **12b** in 85% yield (Scheme 9).

Scheme 9



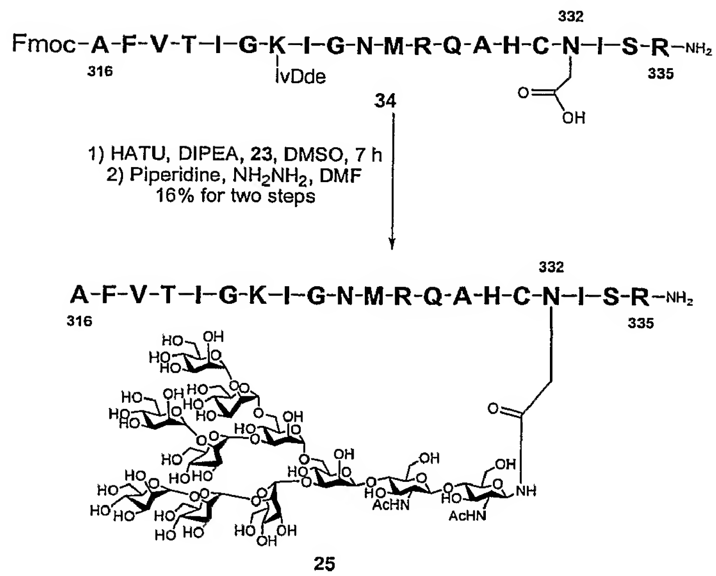
In one example, protected undecasaccharide **12b** was treated with sodium methoxide and HF-pyridine to remove the acetyl groups and TBS group, respectively. The resulting oligosaccharide **21** was then subjected to global Birch deprotection followed by selective acetylation using acetyl anhydride in saturated sodium bicarbonate solution to give free glycan in high yield. Following Kochetkov amination furnished free glycosylamine (Scheme 10).

Scheme 10



In certain embodiments, 20-mer peptide acid **34**, which was made through applied biosynthesis synthesizer, was activate using HATU and coupled directly with glycosylamine **23**. The Fmoc and ivDde protecting groups were removed by treatment with hydrazine and piperidine to give glycopeptide fragment **25** in 16% two steps yield (Scheme 11).

Scheme 11



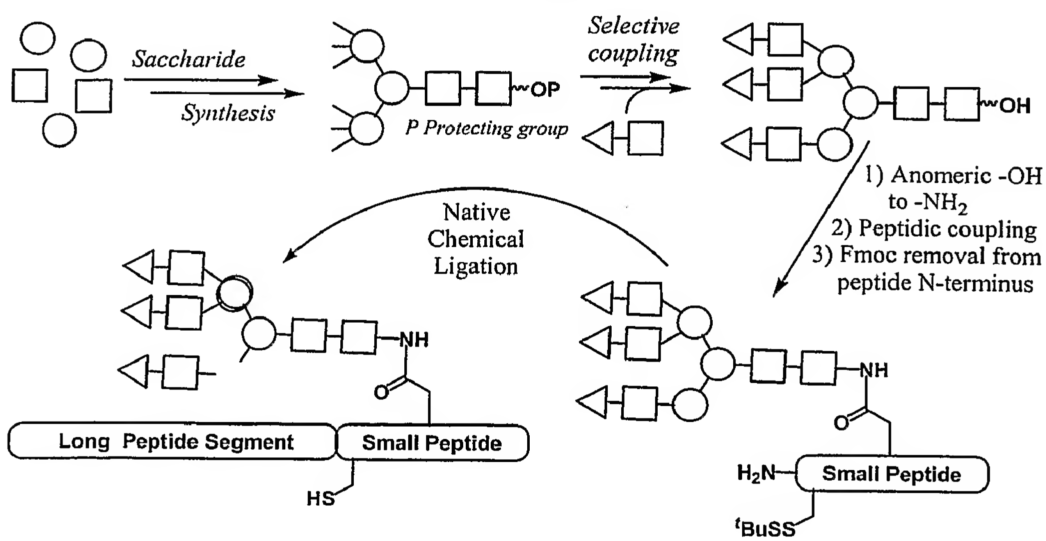
Methods of preparing trisaccharide 3 are known in the art. **(See**, Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. Tetrahedron Letters **2003**, 44, 1791-1793; Zhang, Y.-M.; Mallet, J.-M.; Sinay, P. *Carbohydrate Research* **1992**, *236*, 73-88; Marra, A.; Mallet, J. M.; Amatore, C.; Sinay, P. *Synlett* **1990**, 572-574; Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *Journal of the American Chemical Society* **2003**, *125*, 3402-3403; Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science (Washington, DC, United States)* **2003**, *300*, 2065-2071; Likhosherstov, L. M.; Novikova, O. S.; Derevitskaya, V. A.; Kochetkov, N. K. *Carbohydrate Research* **1986**, *146*, C1-C5)

Glycopeptides

Automated peptide synthesis is reliable for sequences up to about 60 amino acid residues in length, but saccharide moieties contained in glycopeptides render their solid phase synthesis less practical. Unlike peptide synthesis, complex glycan and glycoconjugate synthesis remains readily accessible only to a few select laboratories (See, for example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736). Syntheses of several natural *O*-linked glycopeptides containing simple glycans have been reported (See, for example, (1) Arsequell, G.; Haurum, J. S.; Elliott, T.; Dwek, R. A.; Lellouch, A. C. "Synthesis of Major Histocompatibility Complex Class-I Binding Glycopeptides." *J. Chem. Soc.-Perkin Trans. 1* **1995**, 1739-1745, (2) Chen, X. T.; Sames, D.; Danishefsky, S. J. "Exploration of modalities in building alpha-O-linked systems through glycal assembly: A total synthesis of the mucin-related F1 alpha antigen." *J. Am. Chem. Soc.* **1998**, *120*, 7760-7769; (3) Macmillan, D.; Bertozzi, C. R. "New directions in glycoprotein engineering." *Tetrahedron* **2000**, *56*, 9515-9525; (4) Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C. H. "Chemoenzymatic synthesis of a PSGL- 1 N-terminal glycopeptide containing tyrosine sulfate and alpha-O-linked sialyl Lewis X." *J. Am. Chem. Soc.* **2000**, *122*, 4241-4242; (5) Ajisaka, K.; Miyasato, M.; Ishii-Karakasa, I. "Efficient synthesis of O-linked glycopeptide by a transglycosylation using endo alpha-N-acetylgalactosaminidase from Streptomyces sp." *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1240-1243; and (6) Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. "Chemical synthesis of lymphotactin: A glycosylated chemokine with a C-terminal mucin-like domain." *Chem. Eur. J.* **2001**, 7, 1129-1132), as have examples of mimetics for *N*-linked glycopeptides (See, for

example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736), and a chemoenzymatic synthesis of an *N*-linked glycopeptide (See, for example, Inazu, T.; Haneda, K.; Mizuno, M. "Synthetic study on N-glycopeptides." *J. Syn. Org. Chem. Jpn.* **1998**, *56*, 210-220), but no chemical synthesis has been reported for a natural *N*-linked glycopeptide with complex glycan and peptide structure. The state of the art for chemically synthesized *N*-linked glycopeptides is exemplified by the pentadecasaccharide *N*-linked to a pentapeptide reported by Wang and coworkers, which was recognized by appropriate antibodies to the H-type blood group antigens present at the glycan nonreducing termini (See, for example, Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732).

Scheme 12. Exemplary synthetic approach for the preparation of gp120 glycopeptides.



In one example, as shown in Scheme 12, the chemical synthesis of inventive glycopeptides may be divided logically into two sections: glycan synthesis (top) and glycopeptide assembly (bottom). At its core, the inventive method would extend the method of Wang, *et al.* (Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732) to include one or more peptide elongation steps after synthesis of a short glycopeptide, allowing entry into the realm of fully elaborated, naturally derived glycoproteins (See, for example, Dawson, P. E.; Kent, S. B. H. "Synthesis of native proteins by chemical ligation." *Annu. Rev. Biochem.* **2000**, *69*, 923-960). In an inventive and important improvement, the glycan is fashioned here in a more convergent manner than previously realized, allowing the strategy to be adjusted in its late stage to accommodate the synthesis of various glycoforms, as illustrated in the next section.

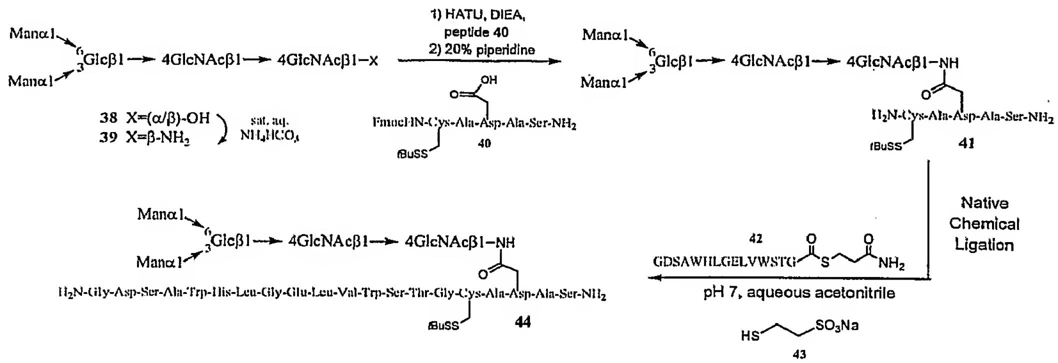
Glycopeptide Assembly

Guidance for glycopeptide assembly may be found, inter alia, in International Application PCT/US03/038471 as it was filed on December 3, 2003; the entire contents of which are hereby incorporated by reference herein. For example, a glycopeptide assembly strategy, as outlined in Scheme 12, involves peptide glycosylation followed by elongation of the peptide backbone, was examined, as illustrated in Scheme 13, using a model peptide and glycan (Miller, J. S. *et al., Angew. Chemie Int. Ed.*, 2003, **42**, 431). To prepare free glycan **38** for coupling, its anomeric hydroxyl was first aminated to give β-aminoglycoside **39** as described by Kochetkov (See, for example,

Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. "A New Simple Synthesis of Amino Sugar Beta-D-Glycosylamines." *Carbohydr. Res.* **1986**, *146*, C1-C5). Glycosylamine **39** and the aspartate free acid of peptide **40** were coupled in peptidic fashion according to the procedure of Lansbury and coworkers ((1) Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537; and (2) Anisfeld, S. T.; Lansbury, P. T. "A Convergent Approach to the Chemical Synthesis of Asparagine- Linked Glycopeptides." *J. Org. Chem.* **1990**, *55*, 5560-5562) with certain modifications: the reported peptide glycosylations involved excess or equimolar amounts of glycosylamine relative to peptide, and their isolated yields (50 - 60%) are reported based on peptide starting material (Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537). As is often the case, however, the saccharide here is the more precious material entering glycosylation because its preparation involves multistep, solution phase synthesis in relatively low overall yield compared to that of the peptide. A trial glycosylation of model pentapeptide **40** with pentasaccharide **39** indicates that under the appropriate reaction conditions, an excess of peptide produces a significantly greater yield of coupled product (over 70% based on valuable glycosylamine) [Miller, J. S. *et al.*, *Angew. Chemie Int. Ed.*, 2003, **42**, 431. Subsequent Fmoc (Fmoc = 9-fluorenylmethyloxy-carbonyl) removal with piperidine afforded glycopeptide **41**.

Scheme 13.

Exemplary glycopeptide assembly route with a model peptide and glycan.

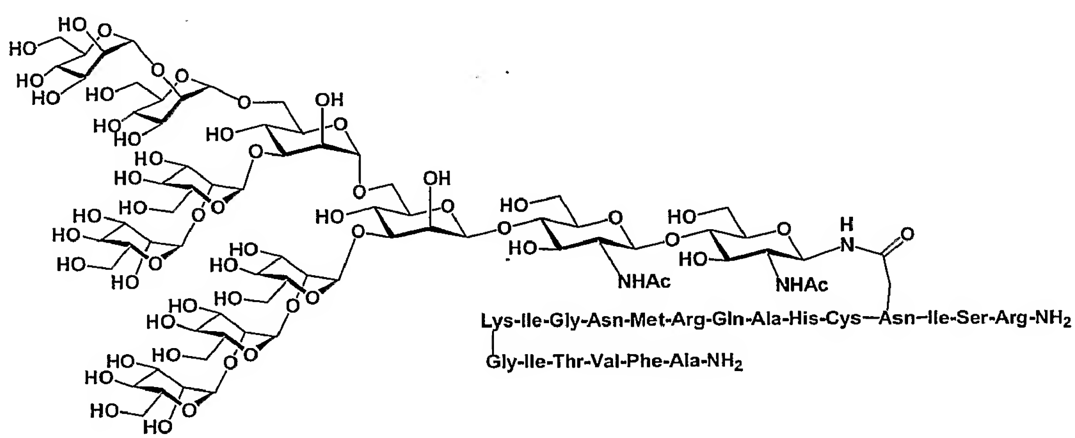


The final step toward completion of a model glycopeptide involved native chemical ligation (NCL) [See, for example, Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. "Synthesis of Proteins by Native Chemical Ligation." *Science* **1994**, *266*, 776-779], as indicated in Scheme 13. *In situ* deprotection of cysteine disulfide **41** and transthioesterification (See, for example, Dawson, P. E.; Churchill, M. J.; Ghadiri, M. R.; Kent, S. B. H. "Modulation of reactivity in native chemical ligation through the use of thiol additives." *J. Am. Chem. Soc.* **1997**, *119*, 4325-4329) of peptide thioester **42** with sodium 2-mercaptoethanesulfonate **(43)** in phosphate-buffered saline (PBS) at neutral pH led to a second thioester exchange with the (now free) cysteine thiol and subsequent rearrangement to give fully unprotected glycopeptide **44**. gp120-derived glycopeptides obtained using the strategy detailed in Scheme 13 will require no additional manipulation other than purification before they can be examined for the generation of antibodies. The synthetic strategy thus requires only four assembly steps starting from free glycans to obtain homogeneous glycopeptides.

The lysine residue may differentially protects with respect to

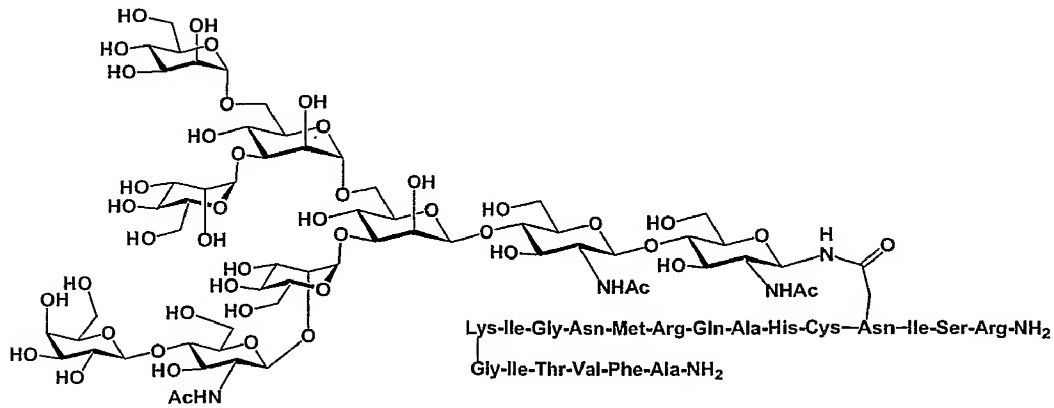
Fmoc removal during peptide synthesis, and remains protected through the peptide glycosylation step (due to its free amine side chain). Suitably protected Lys derivatives have been designed (See, for example, Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. "An appraisal of new variants of Dde amine protecting group for solid phase peptide synthesis." *Tetrahedron Lett.* **1998**, *39*, 1603-1606), and can be deprotected in the presence of *N*-linked saccharides along with the N-terminal Fmoc amine in minutes using hydrazine at room temperature.

In one example, the polypeptide "X" has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



wherein R may be $-(CH_2)_2C(=O)NH_2$.

In another example, the polypeptide "X" has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



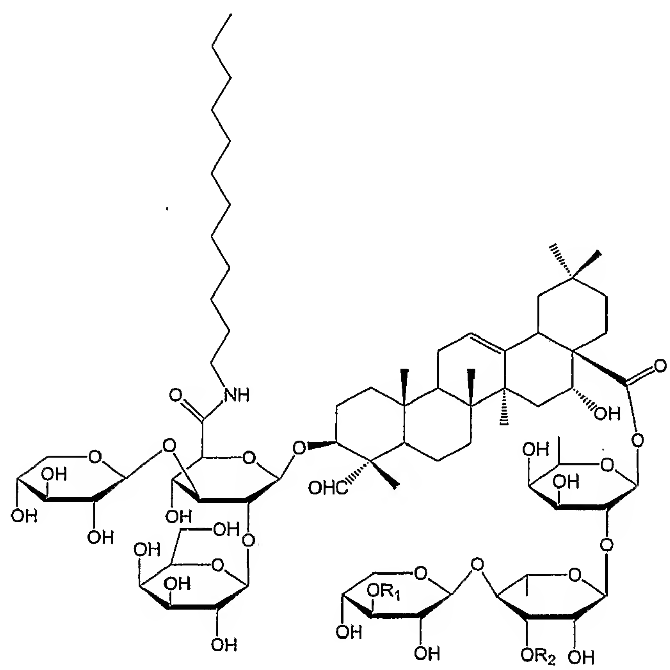
wherein R may be $-(CH_2)_2C(=O)NH_2$.

It will be appreciated that for each of the methods as detailed herein, the full arsenal of protecting groups known in the art of organic synthesis can be utilized, for example, as set forth in "Activating Agents and Protecting Groups: Handbook of Reagents for Organic Synthesis" Roush, W.R. and Pearson, A.J., Eds., John Wiley & Sons: 1999; and "Protective Groups in Organic Synthesis" Greene, T.W. and Wuts, P.G., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference. In but a few examples, suitable protecting groups utilized herein include, but are not limited to, Bn (benzyl), TIPS (triisopropylsilyl), and Ac (acetate). In a certain exemplary embodiments of the present invention, coupling of glycoside moieties are effected under MeOTf promotion, as described herein. It will be appreciated by one of ordinary skill in the art however, that a variety of conditions known in the art of organic synthesis can be utilized to effect coupling of glycoside moieties.

One skilled in the art knows how to adapt the synthetic methods detailed in the present invention to access a variety of other multi-branched gp120 glycans and constructs thereof. In certain other examples, the construct should be so

functionalized as to anticipate the need for its conjugation to an immunogenic carrier (*e.g.*, protein or lipid) in anticipation of the need to stimulate an immune response. Such constructs may be used to generate antibodies for use in HIV vaccine. The present invention provides improvements HIV therapy. The present invention provides novel compounds and compositions for use in HIV therapy.

In certain embodiments, the inventive compositions may comprise an adjuvant. In certain embodiments, the adjuvant is a saponin adjuvant (see, *e.g.*, Marciani *et al.*, *Vaccine*, **2000**, *18*, 3141, US Patent No.: 6,080,725 and 5,977,081, the entire contents of which are hereby incorporated by reference). One example of a preferred saponin adjuvant includes, but is not limited to, GPI-0100, (Galenica Pharmaceuticals, Inc., Frederick, MD) which is a semi-synthetic adjuvant derived by modifying selected natural saponins.



GPI-0100

Saponins isolated from *Quillaja soponaria Molina* contain two

acyl moieties, a normonoterpene carboxylic acid and a normonoterpene carboxylic acid glycoside, which are linked linearly to a fucosyl residue attached at position C-28. It has been hypothesized that these lipophilic acyl groups may be responsible for these saponins' toxicity and their ability to stimulate cytotoxic T cells against exogenous antigens. The linkage between the fucosyl residue and the acyl group is unstable and hydrolyzes under mild conditions (pH≥6) with concomittant loss of saponins capability to stimulate cell-mediated immune response. Unlike their saponin precursors, GPI-0100 adjuvants comprise a stable non-toxic lipophilic moiety in the saponin's glucuronic residue. Methods for preparing these semi-synthetic adjuvants are well-known in the art. For example, GPI-0100 adjuvants may be prepared by hydrolizing quillaja saponins (which are commercially available) under basic conditions to yield the corresponding deacylated product. The deacylated intermediate may then be reacted with a suitable amine reagent using standard carboxylic acid moiety activation methodology to give the desired compounds. A wide variety of procedures are effective for extrating saponin compounds. They are generalized as follows: (i) defatting of the organic matter with a hydrophobic organic solvent such as petroleum ether; (ii) extraction with a suitable alcohol (*e.g.*, methanol or ethanol) or alcohol-water mixture; (iii) evaporation of the carinol solvent; and (iv) partitioning of the dried alcohol extract between water and n-butanol saturated with water, followed by precipitation of the crude saponins from the n-butanol/water with a suitable organic solvent (*e.g.*, diethyl ether). Purification of the saponin extract may require multiple separation steps. For example, preliminary fractionation may be carried out using conventional open column chromatography or flash chromatography on silica gel, in combination with a

more sophisticated chromatographic technique such as High Pressure Liquid Chromatography (HPLC), droplet counter-current liquid chromatography (DCCC) or centrifugal Liquid Chromatography (RLCC). The integration of these techniques with preparative TLC typically affords separated and purified saponins.

In other embodiments, the adjuvant is bacteria or liposomes. In certain examples, the adjuvant includes but is not limited to, *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

In other embodiments, the adjuvant is unmethylated CpG oligonucleotides, alum, Montanide ajuvants, Ribi, PLGA, Enhanzyn, TiterMax adjuvant. These adjuvants are readily commercially available. In other embodiments, the adjuvant is designed to preferentially stimulate mucosal immunity. These include, but are not limited to, cholera toxin and *E. Coli* heat-labile toxin.

Adjuvant therapies may reduce the rate of progression of HIV and/or prevent the onset of HIV.

Thus, the present invention provides pharmaceutical compositions for treating HIV, and for preventing the onset or progression of HIV, comprising any of the compounds of the present invention disclosed herein, as an active ingredient, optionally, though typically in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention may further comprise other therapeutically active ingredients. The inventive compositions include those suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting powders), parenteral (including

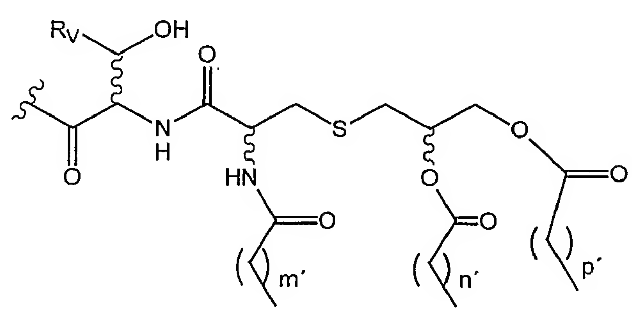
subcutaneous, intramuscular, and intravenous), ocular (opthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy. In certain embodiments, the compositions are suitable for parenteral administration. In certain exemplary embodiments, the compositions are suitable for intravenous administration.

In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (*e.g.*, suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disinterating agents, etc., in the case of oral solid preparations are preferred over liquid oral preparations such as powders, capsules and tablets. If desired, capsules may be coated by standard aqueous or non-aqueous techniques. In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices.

Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the

methods known in the art of pharmacy. In general, compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers, finely divided solid carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granule optionally mixed with a binder, lubricant, inert diluent or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

In one aspect of the invention, a method of eliciting an immune response against HIV-1 or an HIV-1 infected cell in a subject is provided which comprises administering to the subject an amount of the above inventive compounds or compositions in an amount effective to elicit the immune response. In certain embodiments, the method utilizes any one of the inventive compounds linked to an immunogenic carrier, which carrier is a protein, peptide or lipid. In certain embodiments, the carrier is Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or

unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

In certain other embodiments, the method comprises a therapeutically effective amount of any of the compounds disclosed herein, in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier.

It will be appreciated that the magnitude of the therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound and its route of administration. In general, the daily dose range for antiHIV activity lies in the range of 0.0001 to 1.0 mg/kg of body weight in a mammal, although the present invention is not intended to be limited by this range.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound disclosed herein. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, etc. routes may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc. In preferred embodiments, the effective dosage is employed using a syringe injection.

It will be appreciated by one of ordinary skill in the art, however, that the most suitable route for administration will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. As

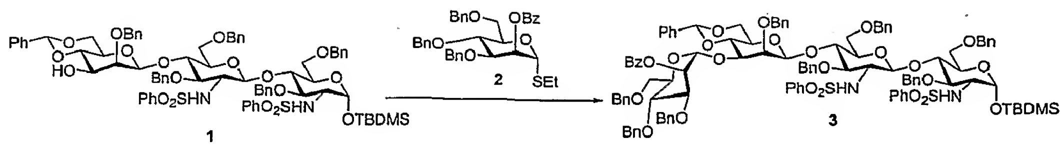
discussed above, the inventive therapeutics may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

Additionally, once a synthetic vaccine has been derivatized and characterized, mouse immunological studies can be performed to assess the potency and/or specificity of the novel HIV vaccines.

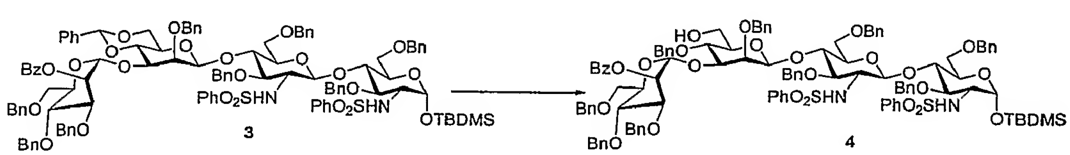
The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in flame-dried glassware under a positive pressure of prepurified argon gas. NMR (^{1}H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^{1}H and 77.0 ppm for ^{13}C) or CD_3COCD_3 (2.09 ppm for ^{1}H and 30.6 and 205.9 ppm for ^{13}C). Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in para-anisaldehyde solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC

plate.

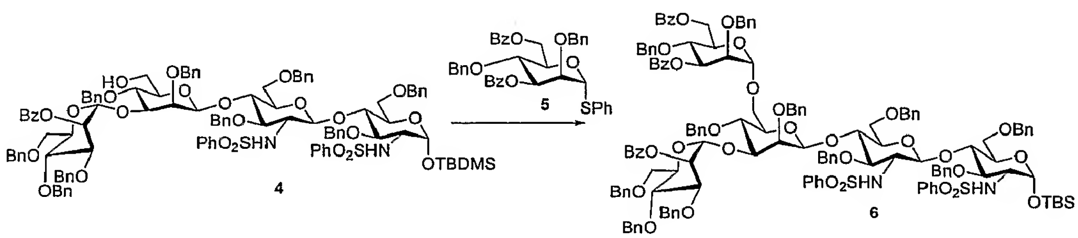


Tetrasaccharide 3: A mixture of trisacchride **1**[1] (106 mg, 0.074 mmol), thiomannoside **2** (133 mg, 0.222 mmol) and molecular sieves in CH_3CN (2 mL) was stirred for 2 h at r.t. and tris(4-bromophenyl)aminium hexachloroantimonate (199 mg, 0.244 mmol) was added at 15 °C. The solution was stirred for 4 h at r.t. and then quenched by triethylamine. The mixture was filtered through celite, concentrated, dissolved in EtOAc, filtered through silica gel and concentrated. The residue was purified by preparative TLC (PTLC) using pentane/ether (1/2) as the eluent to afford **3** as a white solid (113 mg, 78%). $[\alpha]_D^{25}$ −205.0 (*c* 0.14, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.06 (s, 3 H), 0.87 (s, 9 H), 5.07 (s, 1 H), 5.30 (s, 1 H), 5.36 (s, 1 H), 5.74 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.8, −4.6, 13.9, 17.8, 20.8, 25.6, 57.8, 58.6, 60.1, 66.8, 67.5, 67.8, 68.2, 68.4, 68.9, 69.5, 71.0, 72.3, 73.1, 73.3, 73.5, 73.9, 74.3, 74.9, 75.1, 75.3, 75.8, 77.3, 77.6, 77.9, 78.2, 79.9, 92.6, 98.4, 100.7, 100.9, 125.6, 126.7, (126.8-129.5), 129.6, 136.9, 137.4, 137.6, 138.0, 138.1, 138.2, 138.3, 140.4, 141.3, 165.1. LRMS (ESI) calcd for $C_{112}H_{122}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1994.76, found 1994.8.

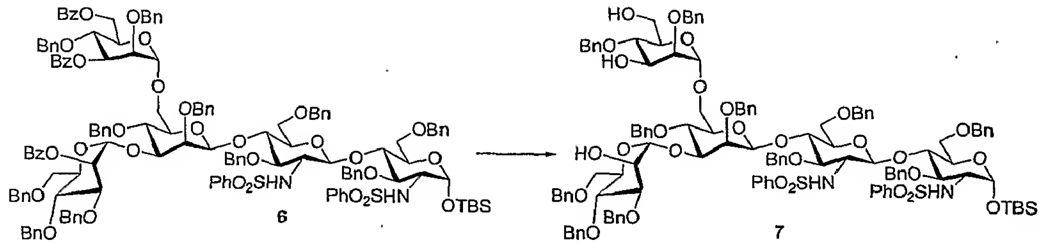


Tetrasaccharide 4: To a solution of **3** (200 mg, 0.101 mmol) in

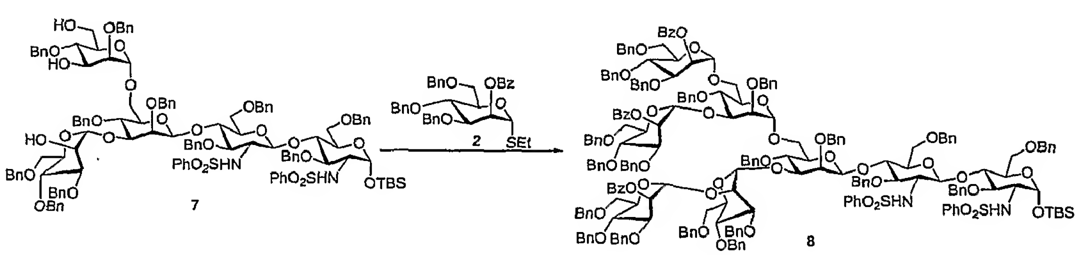
borane tetrahydrofuran etherate (1.1 mL, 1.0 M in THF, 1.01 mmol) was added dibutylboron triflate (0.334 mL, 1.0 M in CH_2Cl_2, 0.333 mmol) at 0 °C. The reaction mixture was stirred for 7 h at 0 °C and quenched with triethylamine and methanol and concentrated. The residue was purified by PTLC using pentane/ether (1/2) as the eluent to afford **4** as a white solid (172 mg, 90%). $[\alpha]_D^{25}$ −187.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ −0.08 (s, 3 H), −0.04 (s, 3 H), 0.80 (s, 9 H), 4.96 (d, *J* = 2.6 Hz, 1 H), 5.15 (s, 1 H), 5.55 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.7, −4.6, 17.9, 25.7, 57.9, 58.3, 67.6, 68.9, 69.8, 71.3, 72.4, 73.2, 73.4, 73.9, 74.3, 74.5, 75.0, 76.0, 77.3, 78.1, 79.6, 79.9, 92.7, 99.3, 100.6, 101.0, 126.8-128.7, 129.8, 137.6, 137.7, 138.2, 138.3, 138.4, 140.5, 141.0, 165.2. LRMS (ESI) calcd for $C_{112}H_{124}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1995.8, found 1995.8.



Pentasaccharide 6: 6 was prepared using same procedure as the synthesis of **3**. White solid (80 mg, 74%). $[\alpha]_D^{25}$ 51.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ −0.07 (s, 3 H), −0.02 (s, 3 H), 0.80 (s, 9 H), 4.95 (s, 1 H), 4.99 (s, 1 H), 5.25 (s, 1 H), 5.54 (dd, *J* = 9.5, 2.5 Hz, 1 H), 5.58 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.8, −4.6, 14.0, 17.9, 20.9, 22.5, 25.7, 31.4, 57.8, 58.6, 60.2, 67.7, 68.9, 69.6, 70.1, 71.5, 72.5, 72.9, 73.2, 73.9, 74.4, 74.8, 75.2, 75.9, 76.4, 77.3, 79.1, 92.7, 97.9, 99.4, 101.1, 126.9-129.5, 129.6, 137.3, 137.7, 138.2, 138.4, 141.1, 165.2, 165.5, 166.1. LRMS (ESI) calcd for $C_{146}H_{154}N_2O_{31}S_2SiNa^+$ $[M+Na]^+$ 2546.0, found 2545.9.

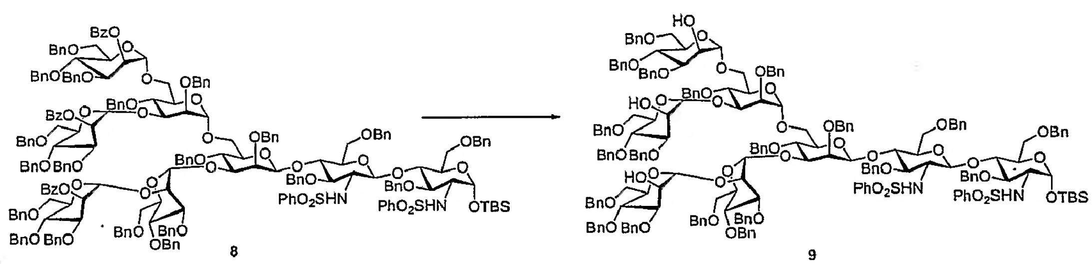


Pentasaccharide triol 7: To a solution of **6** (80 mg, 0.032 mmol) in MeOH (2 mL) was added sodium methoxide in MeOH (25%, 0.1 mL) and stirred for 12 h and quenched with NH_4Cl saturated aqueous solution and concentrated. The residue was dissolved in EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by PTLC using pentane/ether (1/3) as the eluent to afford **7** as a white solid (64 mg, 91%). $[\alpha]_D^{25}$ 121.8 (*c* 0.16, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.93 (s, 9 H), 4.92 (s, 1 H), 5.06 (d, *J* = 1.8 Hz, 1 H), 5.14 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 14.0, 17.9, 20.9, 25.7, 57.8, 58.4, 60.2, 65.9, 68.4, 69.6, 71.6, 71.9, 72.4, 72.6, 73.1, 73.2, 73.3, 73.9, 74.1, 74.4, 74.7, 74.8, 74.9, 75.3, 75.8, 76.1, 76.4, 77.3, 78.3, 79.1, 79.6, 80.6, 92.7, 97.2, 100.5, 101.2, 101.3, 126.8-128.6, 137.6, 137.8, 137.9, 138.2, 138.3, 138.6, 140.5, 141.1. LRMS (ESI) calcd for $C_{125}H_{142}N_2O_{28}S_2SiNa^+$ $[M+Na^+]$ 2233.9, found 2233.9.

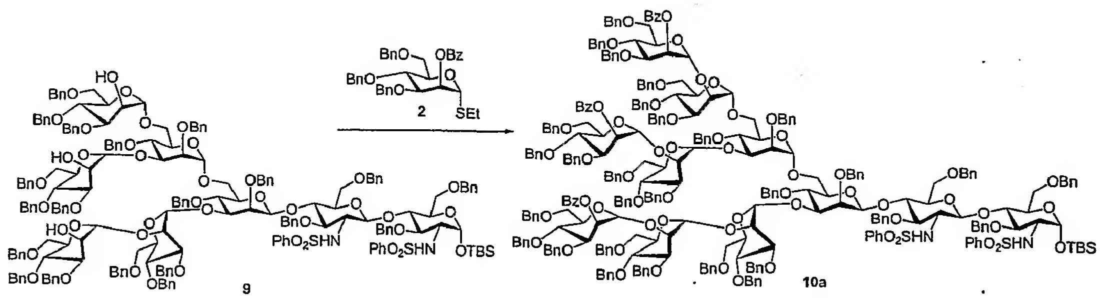


Octasaccharide 8: 8 was prepared following the same protocol

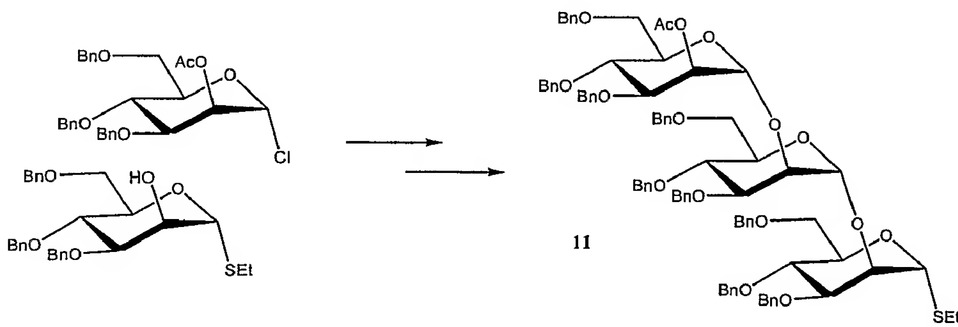
as used for **3** using thiol mannoside donor **2** as excess (10 eq.) White solid: (61 mg, 55%). $[\alpha]_D^{25}$ 32.8 (*c* 0.15, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.88 (s, 9 H), 4.79 (s, 1 H), 4.88 (s, 1 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.23 (s, 1 H), 5.58 (s, 1 H), 5.62 (s, 1 H), 5.66 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -3.9, 14.7, 18.5, 21.5, 26.3, 30.2, 58.4, 59.1, 60.9, 66.5, 69.0, 69.3, 69.4, 70.3, 71.3, 72.0, 72.2, 72.6, 72.8, 73.6, 73.7, 73.8, 74.0, 74.6, 75.1, 75.3, 75.5, 75.7, 78.4, 78.9, 80.4, 82.2, 93.3, 98.2, 98.9, 99.7, 101.2, 101.8, 102.8, 126.9-130.5, 138.5, 138.6, 138.8, 139.0, 139.1, 139.6, 141.2, 165.8, 165.9. LRMS (ESI) calcd for $C_{227}H_{238}N_2O_{46}S_2SiNa_2$ $[M+2Na]^{2+}$ 1932.8, found 1933.0.



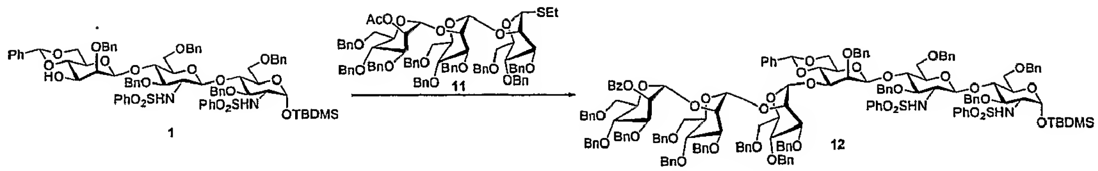
Octasaccharide triol 9: The synthesis of 9 follows the synthetic procedure of **7**. White solid (46 mg, 87%). $[\alpha]_D^{25}$ 280.0 (*c* 0.12, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.08 (s, 3 H), -0.03 (s, 3 H), 0.80 (s, 9 H), 4.92 (s, 1 H), 4.94 (s, 1 H), 4.97 (s, 1 H), 5.03 (s, 1 H), 5.07 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.1, 18.0, 22.7, 25.8, 29.3, 29.7, 31.9, 58.0, 58.6, 65.5, 66.3, 67.7, 68.4, 68.5, 68.8, 71.1, 71.2, 71.7, 71.8, 72.0, 72.3, 72.9, 73.2, 73.3, 73.5, 74.1, 74.2, 74.3, 74.5, 74.8, 74.9, 75.0, 79.4, 81.8, 92.8, 97.4, 100.0, 100.7, 100.9, 101.3, 102.9, 127.1-128.8, 138.0-138.5, 140.7, 141.3. LRMS (ESI) calcd for $C_{206}H_{226}N_2O_{43}S_2SiNa_2$ $[M+2Na]^{2+}$ 1776.7, found 1776.7.



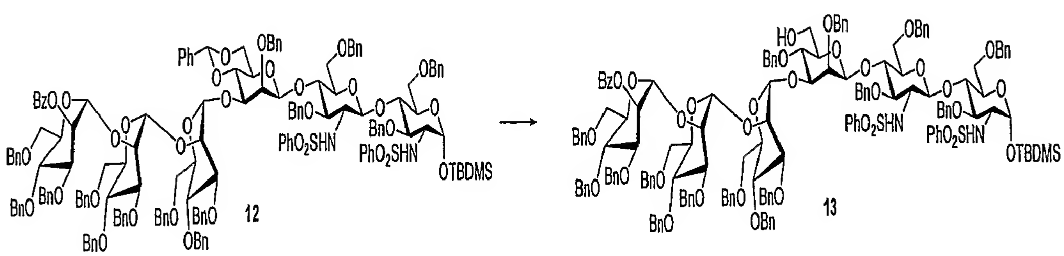
Undecasaccharide 10a: The synthesis of **10a** follows same synthetic procedure as **8**. **10a**, white solid (81 mg, 51%). $[\alpha]_D^{25}$ 73.8 (*c* 0.09, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.05 (s, 3 H), -0.00 (s, 3 H), 0.82 (s, 9 H), 5.00-5.20 (m, 7 H), 5.65-5.68 (m, 3 H). LRMS (ESI) calcd for $C_{308}H_{322}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2581.1, found 2581.3.



Trisaccharide donor 11: Trisaccharide donor **11** was prepared from the known chloride and thiomannoside monosaccharides according to standard coupling procedures. ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.21 (t, *J* = 7.5 Hz, 3H), 2.16 (s, 3H), 2.47-2.57 (m, 2H), 3.57 (d, *J* = 10.7 Hz, 1H), 3.67-3.75 (m, 4H), 3.78-3.85 (m, 4H), 3.89-3.97 (m, 3H), 3.99-4.04 (m, 2H), 4.08-4.12 (m, 3H), 4.36 (d, *J* = 12.2 Hz, 1H), 4.43-4.73 (m, 14H), 4.82-4.88 (m, 3H), 5.08 (d, *J* = 2.0 Hz, 1H), 5.20 (d, *J* = 2.0 Hz, 1H), 5.46 (d, *J* = 1.3 Hz, 1H), 5.56 (dd, *J* = 3.0, 1.9 Hz, 1H), 7.14-7.38 (m, 45H).

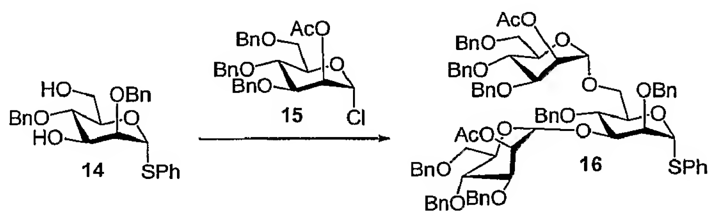


Hexasaccharide 12: To a mixture of **1** (35 mg, 0.024 mmol), **11** (51 mg, 0.037 mmol) and molecular sieves in CH_2Cl_2 (2 mL) was added di-*tert*-butylpyridine (DTBP) (0.019 mL, 0.085 mmol) at -40 °C and stirred for 1 h at -40 °C. MeOTf (0.011 mL, 0.096 mmol) was added and the reaction mixture was warmed up to r.t. and stirred for 12 h before quenched with triethylamine, filtered through celite, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (1/1.3) as the eluent to afford **12** as a white solid (47 mg, 47%). $[\alpha]_D^{25}$ 41.4 (*c* 0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.91 (s, 9 H), 4.97 (s, 1 H), 5.11 (s, 1 H), 5.21 (s, 1 H), 5.25 (s, 1 H), 5.28 (s, 1 H), 5.52 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 25.8, 58.0, 58.8, 60.4, 67.0, 68.6, 68.7, 69.8, 71.6, 71.9, 72.1, 72.9, 73.3, 73.7, 73.8, 74.4, 74.6, 75.1, 75.4, 75.5, 76.0, 77.4, 78.3, 78.8, 80.2, 92.9, 99.5, 99.9, 100.2, 100.8, 101.0, 101.1, 125.9, 127.1-128.5, 137.9, 138.1, 138.4, 138.6, 138.8, 140.7, 170.0, 171.1. LRMS (ESI) calcd for $C_{161}H_{176}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1409.6, found 1409.4.



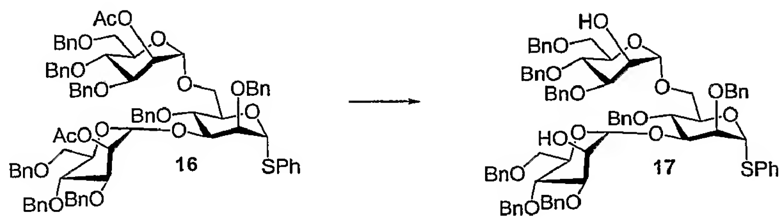
Hexasaccharide 13:13 was prepared using the same procedure as

the one for **4**. **13**, white solid (542 mg, 86%). $[\alpha]_D^{25}$ 91.5 (*c* 0.54, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.02 (s, 3 H), 0.04 (s, 3 H), 0.87 (s, 9 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.09 (s, 1 H), 5.15 (s, 1 H), 5.49 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 21.4, 25.8, 58.0, 58.3, 60.4, 61.3, 67.6, 68.6, 69.9, 71.8, 72.0, 72.1, 73.1, 73.3, 73.5, 74.1, 74.5, 74.6, 74.8, 75.1, 76.1, 76.2, 78.2, 78.7, 79.8, 81.1, 92.8, 99.4, 100.5, 100.7, 101.0, 101.3, 125.3, 127.0-128.5, 137.9, 138.0, 138.4, 138.5, 138.6, 138.7, 140.7, 170.1. LRMS (ESI) calcd for $C_{161}H_{178}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1410.6, found 1410.4.



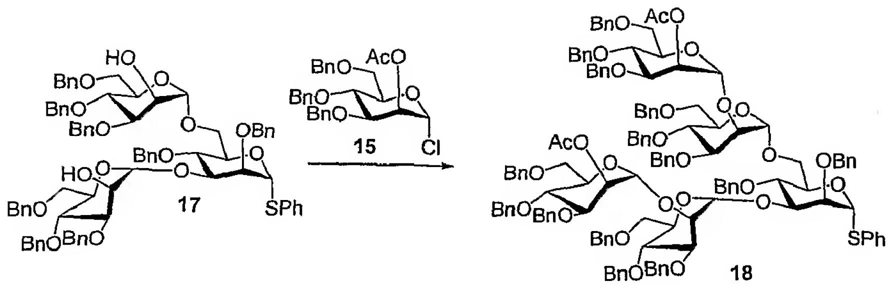
Tirsaccharide 16: To a 25 mL flask containing donor **15** (169 mg, 0.332 mmol) and acceptors **14** (37 mg, 0.083 mmol) (dried azeotropically with toluene) in 1.5 mL dichloromethane was added activated MS 4Å and the mixture was stirred for 1 h at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL dichloromethane were stirred with MS 4Å. After one hour the flask containing the AgOTf / DTBP was cooled to -10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with warming up to room temperature over 18 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$ solution. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column

chromatography (10% ethyl acetate/toluene) to afford diacetate **16**. This diacetate was used for next step without further purification.

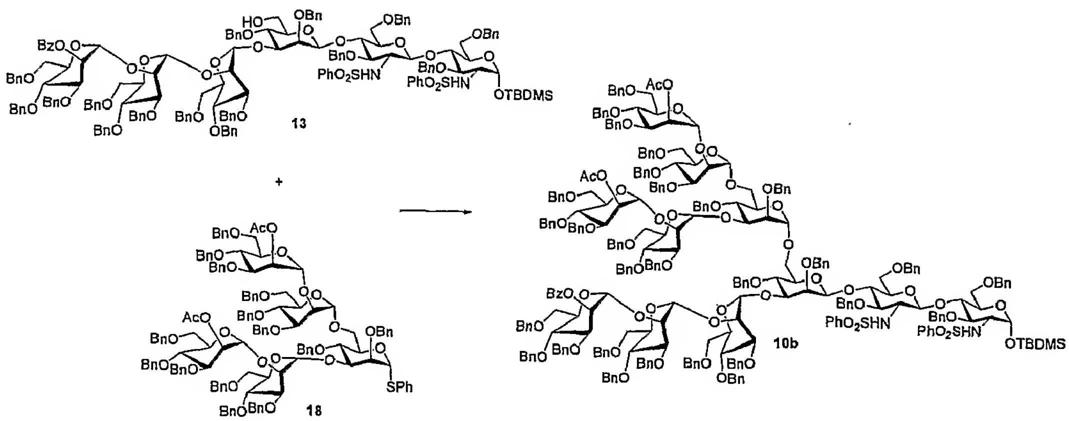


Trisaccharide diol 17:

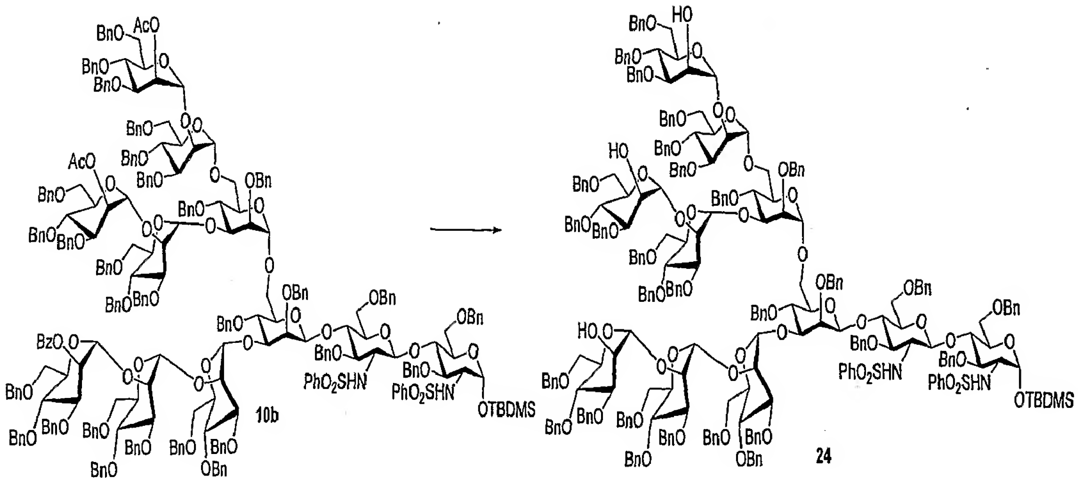
16 was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide (25% by weight in methanol, 100 μL) was added and the reaction mixture was stirred for 12 h. Solid ammonium chloride was added and the mixture was stirred for 20 min. The reaction mixture was carefully evaporated to solid residues, and the residues were dissolved in ethyl acetate and washed with brine. Evaporation of ethyl acetate layers provided crude products, which was purified by silica gel column chromatography (10% ethyl acetate/dichloromethane) to yield diol **17** in 50% over two steps. $[\alpha]_D^{25}$ +53.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.94 (bs, 1H), 5.17 (bs, 1H), 5.44 (bs, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. LRMS (ESI) calcd for $C_{80}H_{84}O_{15}SNa^+$ $[M+Na]^+$ 1339.6, found 1339.5.



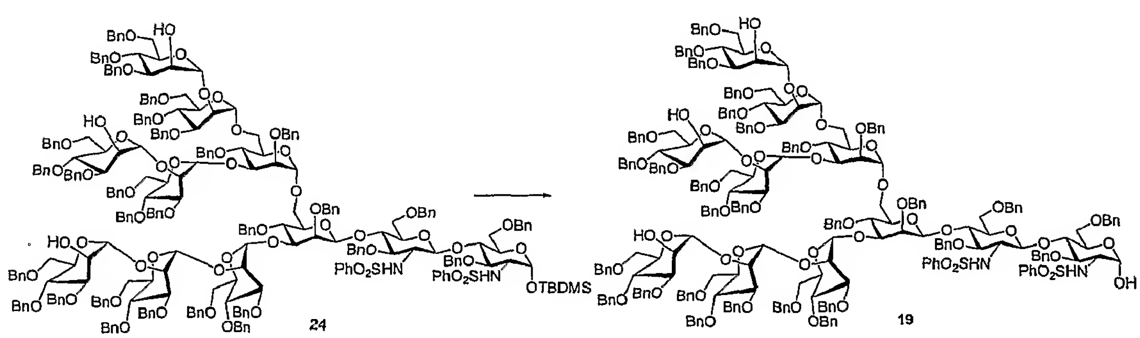
Pentasaccharide 18: To a mixture of **17** (208 mg, 0.158 mmol), **15** (332 mg, 0.631 mmol), molecular sieves, DTBP (0.088 mL, 0.347 mmol) in CH_2Cl_2 (13 mL) was added AgOTf (166 mg, 0.646 mmol) at 0 °C. The mixture was stirred for 18 h at r.t. and quenched with triethylamine, filtered, diluted with EtOAc, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (2/1) as the eluent to afford **18** as a white solid (310 mg, 87%). $[\alpha]_D^{25}$ 443.4 (*c* 0.49, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 2.10 (s, 3 H), 2.11 (s, 3 H), 4.88 (s, 1 H), 5.02 (s, 1 H), 5.04 (s, 1 H), 5.21 (s, 1 H), 5.51 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.9, 21.0, 21.1, 29.6, 44.6, 60.3, 66.6, 68.7, 68.8, 71.5, 71.7, 71.8, 72.0, 72.1, 73.1, 73.3, 73.4, 74.1, 74.2, 74.4, 74.6, 74.7, 75.0, 75.2, 78.0, 78.1, 79.2, 80.3, 84.8, 89.8, 95.4, 99.0, 99.4, 99.5, 101.2, 116.9, 125.1, 127.1-128.4, 129.1, 130.8, 138.0-138.6, 146.8, 168.3, 170.0. LRMS (ESI) calcd for $C_{138}H_{144}O_{27}SNa^+$ $[M+Na]^+$ 2288.0, found 2287.9.



Undecasaccharide 10b: The preparation of **10b** from **18** and **13** follows the same procedure as the one used for **3**. **10b**, white solid (529 mg, 63% yield, 85% based on recovered starting material). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.07 (s, 3 H), 0.15 (s, 3 H), 0.90 (s, 9 H), 2.01 (s, 3 H), 2.10 (bs, 6 H), 5.05 (bs, 1 H), 5.07 (bs, 1 H), 5.10 (bs, 1 H), 5.12 (bs, 1 H), 5.13 (bs, 1 H), 5.15 (bs, 1 H), 5.23 (bs, 1 H), 5.51 (bs, 1 H), 5.54 (bs, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 1.0, 14.2, 18.0, 21.0, 21.1, 21.2, 25.8, 29.7, 58.0, 58.6, 60.4, 68.6, 68.7, 68.8, 71.8, 72.2, 72.3, 73.0, 73.1, 73.2, 73.3, 74.2, 74.5, 74.8, 75.0, 75.1, 78.2, 78.3, 78.4, 79.4, 92.8, 99.3, 99.5, 100.7, 101.6, 102.3, 127.3-128.4, 138.1-138.7, 140.7, 141.3, 170.0, 170.1, 170.15. LRMS (ESI) calcd for $C_{293}H_{316}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2488.0, found 2488.0.

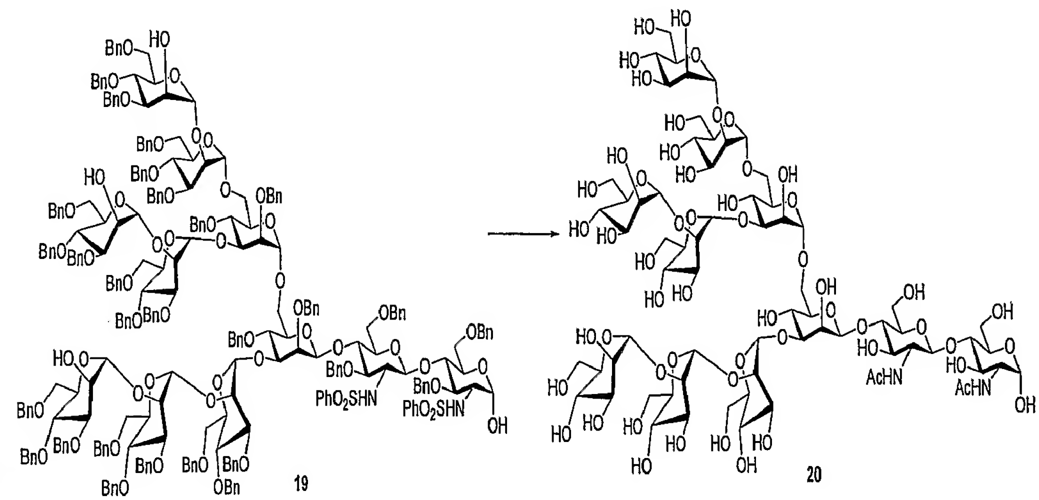


Undecasaccharide triol 24: 24 was prepared using the same procedure as described for **7. 24**, white solide (468 mg, 96%). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.03 (s, 3 H), 0.05 (s, 3 H), 0.90 (s, 9 H), 5.07 (s, 1 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.18 (s, 1 H), 5.21 (s, 1 H), 5.30 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.5, 0.0, 14.1, 18.0, 21.0, 25.8, 29.6, 57.9, 58.6, 60.3, 67.6, 68.4, 68.6, 68.7, 68.9, 71.5, 71.9, 72.0, 72.3, 73.0, 73.1-73.5, 74.2, 74.5, 74.7, 74.9, 75.0, 75.2, 79.9, 80.0, 92.7, 99.4, 100.2, 100.7, 101.1, 101.5, 102.3, 126.7-128.7, 138.1-138.8, 140.7, 141.3. LRMS (ESI) calcd for $C_{287}H_{310}N_2O_{58}S_2SiNa_2$ $[M+2Na]^{2+}$ 2425.0, found 2425.2.



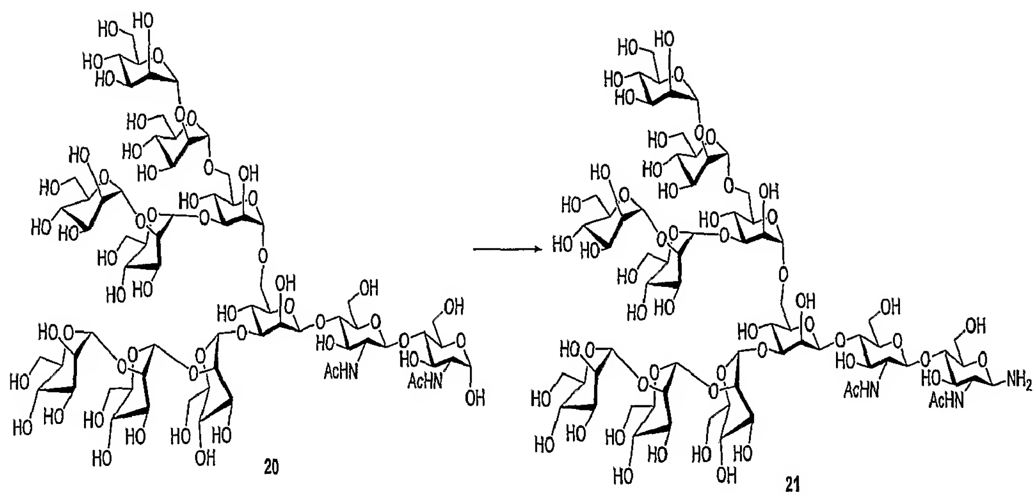
Undecasaccharide tetraol 19: To a solution of **24** (468 mg, 0.097 mmol) in HAc (1.0 M in THF, 2.5 mL) was added TBAF (1.0

M in THF, 2.5 mL) and the reaction mixture was stirred for 1 h before additional HAc (1.0 M in THF, 5.0 mL) was added. The mixture was concentrated and residue purified by column chromatography using 2.5% MeOH in CH_2Cl_2 as the eluent to afford **19** as a white solid (460 mg, 98%). $[\alpha]_D^{25}$ 121.7 (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.95 (bs, 2 H), 5.08 (s, 1 H), 5.20 (bs, 2 H), 5.24 (s, 1 H), 5.27 (s, 1 H). LRMS (ESI) calcd for $C_{281}H_{296}N_2O_{58}S_2Na_2$ $[M+2Na]^{2+}$ 2367.9, found 2367.6.

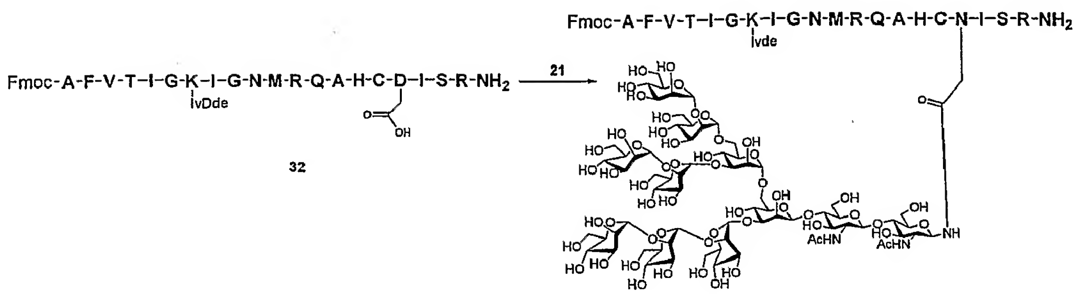


Glycan 20: To a solution of sodium (101 mg, 4.391 mmol) in 15 mL liquid ammonia was added **19** (95 mg, 0.020 mmol) in THF (4 mL) at -78 °C and the reaction mixture was stirred for 2 h at -78 °C. The reaction was quenched with solid NH_4Cl at -78 °C and then warmed up to r.t. while argon was blowing through the reaction flask to evaporate all liquid. The residue was dried on vaccum for 2 h and dissolved in saturated $NaHCO_3$ aqueous solution (2 mL) and cooled to 0 °C. Ac_2O (0.1 mL) was then added at 0 °C and the ice bath was then removed and 5 min later additional Ac_2O (0.05 mL) was added. 30 min later, low resolution mass spectrum showed reaction is complete. The reaction mixture was loaded on to a Bio-Gel P-2 column (BIO-

RAD, catalog number 150-4134, molecular cutoff 2000) using water as the eluent to remove salt and small molecular weight compounds. The fraction containing desired material (illustrated by MassSpectrum) was conbined and lyophilized to afford glycan **20** as a white solid (33 mg, 87% from **19**). ^{1}H NMR (400 MHz, $CDCl_3$) selected signals: δ 5.07 (bs, 2 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.33 (s, 1 H), 5.36 (s, 1 H), 5.40 (s, 1 H). LRMS (ESI) calcd for $C_{70}H_{118}N_2O_{56}Na^+$ $[M+Na]^+$ 1905.6, found 1905.6.



Glycosylamine 21: A solution of **20** (33 mg, 0.018 mmol), NH_4Cl (10 g) in 30 mL water was heated to 40 °C for 2 days and Mass spectrum indicated that reaction is complete. So the reaction mixture was frozen and lyophilized. The residue was dissolved in 20 mL water, frozen and lyophilized again. This process was repeated until the weight of the residue is constant (36 mg). LRMS (ESI) calcd for $C_{70}H_{119}N_3O_{55}Na^+$ $[M+Na]^+$ 1904.7, found 1904.8.



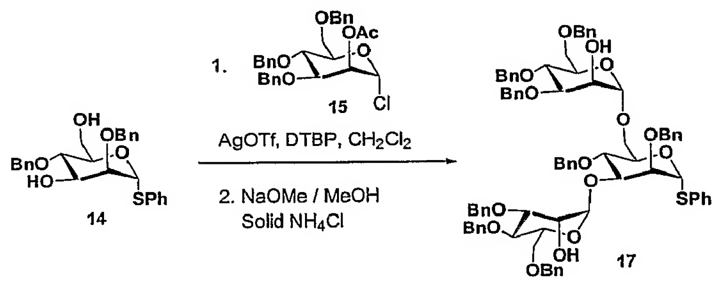
32

23

Gp120 glycopeptide 23: A solution of peptide acid **32** (21 mg, 0.008 mmol), HATU (6 mg, 0.016 mmol), diehtylpropylamine (DIEPA) (2 µL, 0.011 mmol) in DMSO (150 µL) was stirred for 5 min and transferred to the flask containing **21** (5 mg, 0.002 mmol) and the reaction mixture was stirred for 2 h. Additional DIEPA was added (0.6 µL at 4 h and 0.6 □L at 6 h). At 7 h, a mixture of hydrazine, piperidine and DMF (volume ratio: 5:15:85, 0.2 mL) was added and the reaction mixture was stirred for 5 min and TFA in water (10%, 0.55 mL) was added and stirred for 30 min. The crude solution was purified by HPLC using a Varian C18-DYNAMAX-60 Å column. HPLC Conditions: 10%B to 50%B over 50 min, UV 214 nM (A: 0.05% TFA in water; B: 0.04% TFA in CH_3CN). Retention time: 19.8 min. The fraction containing **23** was lyophilized to give **23** as a white solid (1.7 mg, 16% from **20**). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.16 (s, 1 H), 5.19 (s, 1 H), 5.25 (s, 1 H), 8.29 (s, 1 H). LRMS (ESI) calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_3$ $[M+3Na]^{3+}$ 1360.6, found 1360.7; calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_4$ $[M+4Na]^{4+}$ 1020.7, found 1020.6.

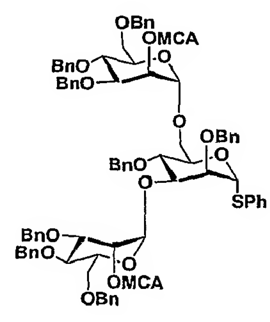
(See, Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *Tetrahedron Letters* **2003**, *44*, 1791-1793; Zhang, Y.-M.; Mallet, J.-M.; Sinay, P. *Carbohydrate Research* **1992**, *236*, 73-88; Marra, A.; Mallet, J. M.; Amatore, C.; Sinay, P. *Synlett* **1990**, 572-574; Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.;

Otake, K.; Kato, K.; Ito, Y. *Journal of the American Chemical Society* **2003**, *125*, 3402-3403; Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science (Washington, DC, United States)* **2003**, *300*, 2065-2071; Likhosherstov, L. M.; Novikova, O. S.; Derevitskaya, V. A.; Kochetkov, N. K. *Carbohydrate Research* **1986**, *146*, C1-C5.)



Into a 25 mL flask containing donor **15** (0.169 gm, 0.332 mmol) and acceptors **14** (0.037 gm, 0.083 mmol) (azeotropically dried with toluene) in 1.5 dichloromethane was added activated MS 4A and the mixture was stirred for 1 hr at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL of dichloromethane were stirred with MS 4A. After stirring for 1 hr, the flask containing the AgOTf / DTBP was cooled to – 10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with gradual warming up to room temperature over 24 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, then with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography

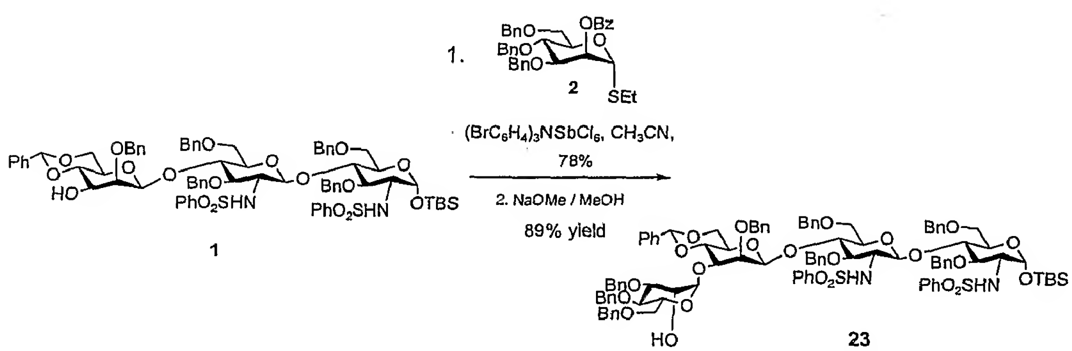
(10% ethyl acetate / toluene) to afford semi pure trimer diacetate. This diacetate was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide, 25% by weight in methanol (100 μL) was added and the reaction mixture was stirred for 12h. Solid ammonium chloride was added and the resulting solution was stirred for 20 min. The reaction mixture was carefully evaporated to solid residues, and the solid residues were washed with ethyl acetate. Evaporation of ethyl acetate layer provided crude product, which was purified by silica gel column chromatography (10% ethyl acetate / dichloromethane) to yield diol **17** in 65% yield (over two steps. [α] + 53.1 (c 1, $CHCl_3$); ^1H – NMR (CDCl$_3$, 400 MHz) δ 7.33-7.03 (45H, m, aromatic), 5.44 (1H, br-s), 5.17 (1H, br-s), 4.94 (1H, br-s), ; ^{13}C-NMR (CDCl$_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. ESI-MS calcd for $C_{80}H_{84}O_{15}S$ Na $[M+Na]^{1+}$ m/z = 1339.5: found 1339.5



22

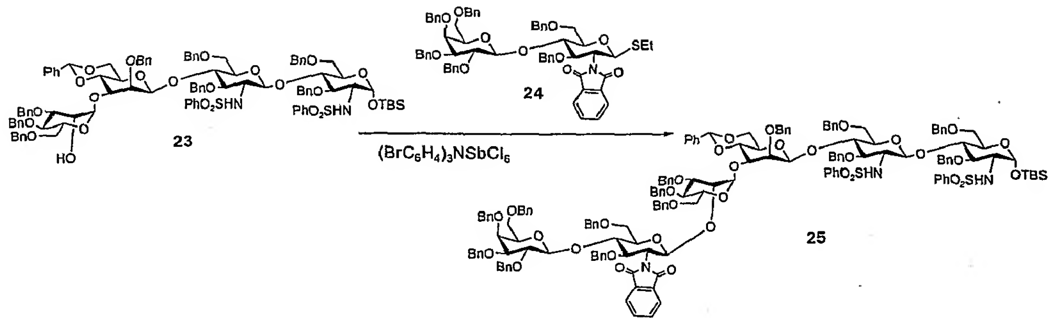
Into a 25 mL flask containing **17** (0.099 gm, 0.0689 mmol,

azeotropically dried with toluene) in 0.4 mL mL of dry dichloromethane under argon and cooled to 0 °C. Pyridine (55 μL, mmol, 6.8 mmol) and chloroacetic anhydride (0.047 gm, .0275 mmol), were added successively and resulting reaction mixture was stirred for 2 h at 0 °C, and then diluted with ethyl acetate, washed two times with 0.5N HCl, water, sat $NaHCO_3$, brine, and dried with $MgSO_4$. Evaporation of ethyl acetate layer followed by silica gel column chromatography (20 ethyl acetate in hexanes) provided 0.166 gm (79% yield) of **22**. R_f 0.33 (20% ethyl acetate in hexanes). [α] + 58.2 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) δ 7.30-7.03 (45H, m, aromatic), 5.47 (2H, m), 5.41 (1H, br-s), 5.15 (1H, s), 4.88 (1H, s), 4.75 (2H, t, J = 10.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.88, 166.78, 138.57, 138.51, 138.3, 137.79, 137.76, 137.74, 134.7, 130.9, 129.3, 128.7, 128.6, 128.59, 128.51, 128.48, 128.43, 128.30, 128.10, 128.0, 127.97, 128.86, 127.78, 127.75, 127.74, 127.7, 127.4, 99.5, 97.9, 84.9, 79.0, 78.1, 77.7, 75.4, 75.2, 75.1, 74.3, 74.1, 73.7, 73.5, 72.43, 72.37, 72.29, 71.78, 71.69, 70.7, 70.4, 69.0, 68.7, 66.8, 41.2, 41.0



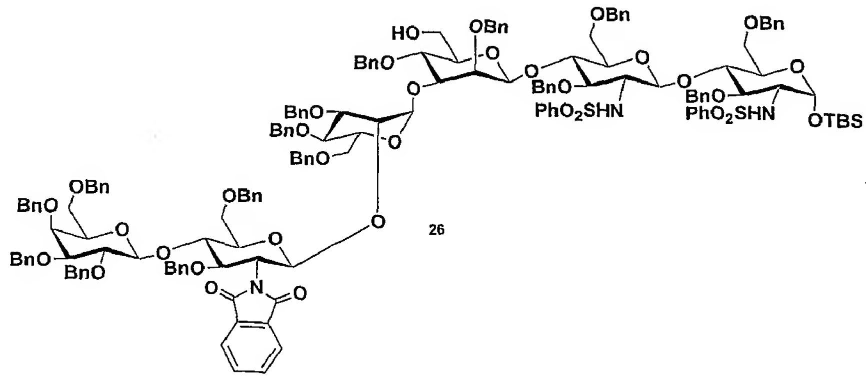
Into a 25 mL flask containing donor **2** (125 mg, 0.0696 mmol, azeotropically dried with toluene) and 4A molecular sieves in dry acetonitrile were stirred for 1 hr under argon. Tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (140 mg, promoter) and then a solution of acceptor **1** (100 mg,

0.0696 mmol) were added slowly while cooling the flask at 15 °C. After stirring for 15 min, another portion of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (46 mg) was added and the reaction mixture was warmed to room temperature and stirred for 3 hr. Freshly distilled triethyl amine (1.5 mL) was added to neutralize the reaction. The reaction mixture was filtered through a bed of Celite and concentrated. The crude product was purified by silica gel column chromatography to afford tetrasaccharide (0.110 gm). R_f 0.65 (20% ethyl acetate in toluene). Under argon this material was dissolved in mixture of dry methanol (2 mL) and dichloromethane (1.5 mL). Sodium methoxide, 25% by weight in methanol (0.038 mL) was added and stirred for 12 hr. Solid ammonium chloride was added and the mixture was evaporated to dryness. The solid residue was washed several times with ethyl acetate and concentrated. Purification by silica gel column chromatography afforded the 0.092 gm (89% yield) of **23**. R_f 0.42 (40% ethyl acetate in hexanes). [α] − 8.8 (c 1, $CHCl_3$); ^1H − NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.75 (2H, d, J = 7.6 Hz), 7.72 (2H, d, J = 6.8 Hz), 5.41 (1H, br-s), 5.26 (1H, d, J = 2.0 Hz), 5.11 (1H, d, J = 2.4 Hz), 3.14 (1H, m), 3.0 (2H, m), 1.57 (1H, br-s), 0.908 (9H, s), 0.09 (3H, s), 0.03 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 141.7, 140.9, 138.7, 138.65, 138.61, 138.4, 138.0, 137.9, 137.8, 137.5, 132.6, 132.4, 129.5, 129.1, 129.0, (128.9-127.6), 127.4, 127.3, 127.2, 126.2, 101.5, 101.3, 101.1, 100.4, 93.0, 80.3, 80.0, 76.2, 75.8, 75.6, 75.5, 75.2, 74.8, 74.4, 73.96, 73.87, 73.6, 72.2, 72.0, 69.9, 69.3, 68.8, 68.5, 68.2, 67.8, 67.2, 37.5, 33.8, 33.6, 32.1, 30.3, 30.2, 29.9, 29.5, 29.1, 27.3, 26.9, 26.0, 23.4, 22.9, 19.9, 18.2, 14.4, 14.3, 7.6, − 4.2, − 5.4;



Into a 25 mL round-bottomed flask containing acceptor **23** (0.100 gm, 0.0535 mmol, azeotropically dried with toluene) in 1 mL dry acetonitrile was added 4A activated molecular sieves and was stirred at room temperature under argon for 1 hr. Similarly the donor **24** (0.1498 mmol, 0.158 gm) and 4A molecular sieves in 1 mL dry acetronitrile were stirred at room temperature for 1 hr. The flask containing donor was cooled to 15 °C and 0.091 gm of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (promoter) was added followed by the solution of acceptor. The reaction mixture was stirred at 15 °C for 20 min and then additional 0.031 gm of promoter was added. The cooling bath was removed and the reaction mixture was stirred for 3 hr. The reaction mixture was cooled to 0 °C and triethyl amine (1 mL) was added. After stirring 10 min at 0 °C, the reaction mixture was warmed to room temperature and stirred for additional 10 min. Filtering through a pad of Celite and evaporation of the filtrate afforded the crude product, which was purified by preparative TLC (first using 5% ethyl acetate in dichloromethane, then 30% ethyl acetate in hexanes) to afford .091 gm of **25** (60% yield), [α] - 16.6 (c 1, $CHCl_3$); ^1H - NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.72 (2H, d, J = 7.2 Hz), 7.67 (2H, d, J = 7.2 Hz), 5.21 (1H, br-s), 5.07 (1H, br-s), 5.01 (1H, br-s), 4.98 (1H, d, J = 8Hz), 2.99 (1H, m), 2.89 (1H, t, J = 8.4 Hz), 2.80 (1H, m), 2.64 (2H, m), 0.875 (9H, s), 0.07 (3H, s), 0.05 (3H, s);

^{13}C-NMR ($CDCl_3$, 100 MHz). δ 140.5, 139.7, 138.04, 138.01, 137.7, 137.69, 137.63, 137.59, 137.56, 137.47, 137.41, 137.3, 137.0, 136.8, 136.5, 136.4, 132.4, 131.3, 131.1, 130.9, 128.4, 128.1, 127.8, (127.5-126.1), 125.8, 125.3, 122.4, 122.0, 102.3, 100.3, 99.9, 99.4, 96.5, 94.7, 91.8, 81.5, 78.9, 77.6, 77.36, 77.31, 75.1, 74.4, 74.2, 74.1, 73.9, 73.8, 73.6, 73.5, 73.1, 72.8, 72.7, 72.4, 72.2, 71.9, 71.8, 71.6, 71.5, 71.2, 57.8, 56.9, 51.3, 28.7, 24.8, 16.9, 7.6, - 5.4, - 6.6

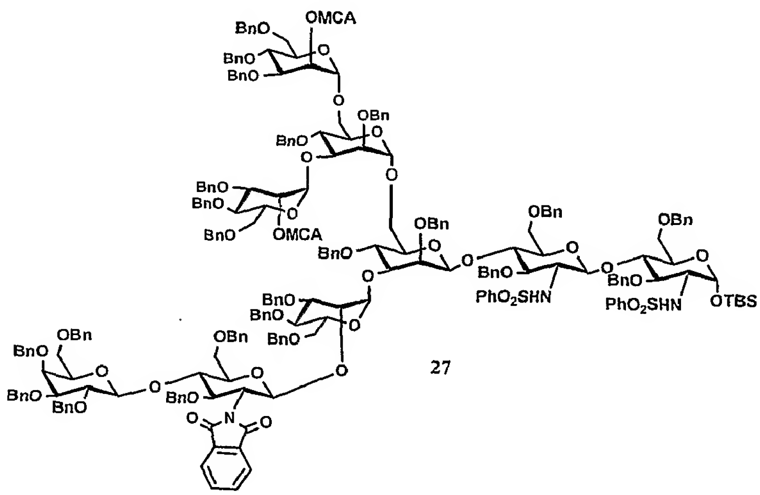


26

To the azeotropically dried **25** (0.307 gm, 0.107 mmol) in 25 mL round bottomed flask was added 8 mL of BH_3.THF (1 molar) and stirred for 5 minutes at room temperature. The reaction mixture was cooled to 0 °C and 0.35 mL of $n$$Bu_2$OTf (1 molar in CH_2Cl_2) was added. The resulting reaction mixture was stirred at 0 °C for 9 hr. Freshly distilled triethyl amine (0.492 mL) was added and followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction was evaporated to dryness, twice codistilled from methanol to afford the crude product as clear oil. Purification by silica gel column chromatography (30% ethyl acetate in hexanes) provided the **26** in 75% yield (0.231 gm).

[α] - 7.0 (c 1, $CHCl_3$); ^{1}H - NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.74 (2H, d, J = 7.2 Hz), 7.69 (2H, d, J = 6.8 Hz), 5.07 (2H, m), 2.96 (1H, m), 2.75 (2H, m), 0.90 (9H, s), 0.07

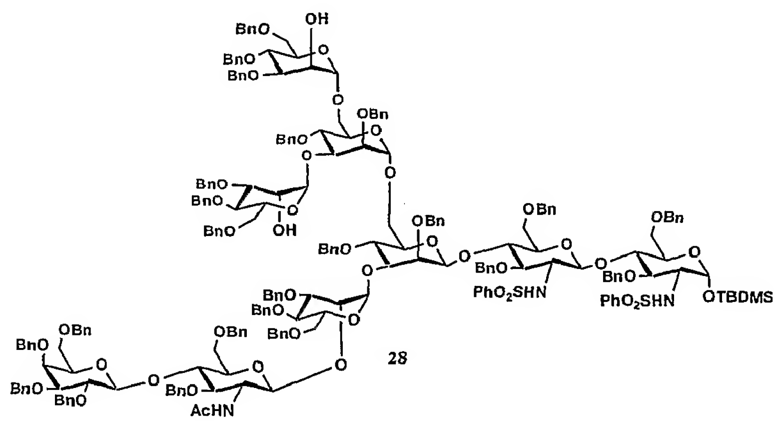
(3H, s), 0.02 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz). δ 168.5, 167.7, 141.4, 140.9, 139.3, 139.2, 138.97, 138.91, 138.73, 138.66, 138.64, 138.58, 138.50, 138.45, 138.27, 138.1, 137.8, (129.2-126.9), 123.5, 123.4, 103.3, 101.0, 100.9, 99.2, 96.5, 92.9, 82.6, 80.1, 79.9, 78.7, 78.4, 76.5, 76.0, 75.9, 75.4, 75.1, 74.9, 74.8, 74.68, 74.63, 74.49, 74.34, 74.29, 74.24, 73.84, 73.77, 73.6, 73.45, 73.3, 73.2, 73.0, 72.8, 72.7, 61.4, 60.6, 58.6, 59.2, 55.8, 26.0, 21.3, 19.3, 18.2, 14.4, 14.1, -4.2, - 5.4;



27

Into a 5 mL v vial were taken azeotropically dried donor **22** and acceptor in **26** mL anhydrous acetonitrile and activated 4A MS was added. The resulting reaction mixture was stirred under argon for 1 hour at room temperature and then was cooled to 15 °C. At this point Tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] was added to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at room temperature for 12 h or TLC indicated the disappearance of the acceptor. The reaction mixture was cooled to 0 °C and 2 mL triethyl amine was added and stirred for 30 minutes with gradual warming up to room temperature. The reaction mixture was filtered through a pad of Celite and

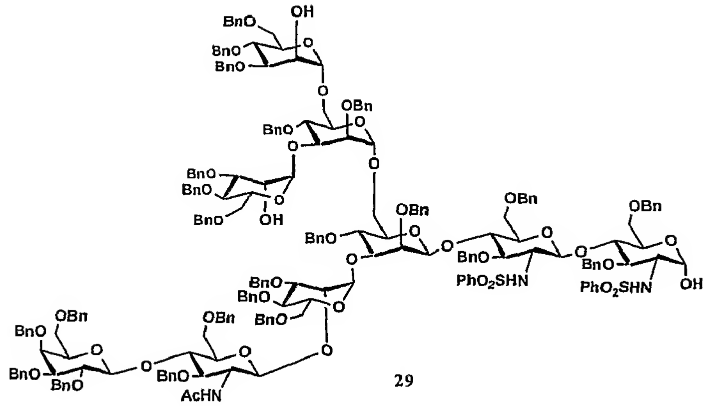
concentrated to provide crude material, which was purified by preparative TLC (20x20cm x 1 mm thickness PK6F plates) using 40% ethyl acetate in hexanes to yield **27**. [α] + 9.4 (c 1, $CHCl_3$); ^1H - NMR ($CDCl_3$, 400 MHz) (selected protons) δ 5.41 (1H, br-s), 5.32 (1H, br-s), 5.09 (1H, br-s), 4.97 (2H, m), 0.83 (9H, s), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.6, 167.6, 166.70, 166.67, 141.5, 140.9, 139.30, 139.23, 139.0, 138.9, (138.8-138.1), 137.89, 137.86, 137.6, 133.6, 132.5, 132.3, 132.0, (129.0-126.9), 126.7, 103.3, 101.7, 100.9, 99.3, 98.0, 97.8, 96.3, 92.9, 82.6, 81.3, (78.9-65.1), 58.6, 58.1, 55.8, 39.9, 39.8, 28.7, 24.8, 17.0, - 5.4, - 6.7.



28

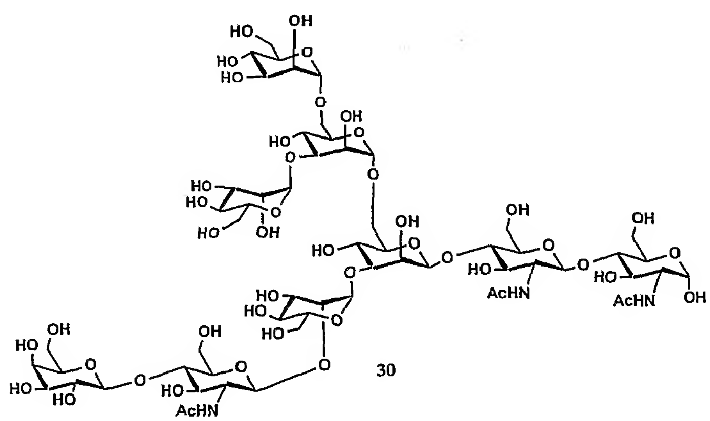
To azeotropically dried **27** (100 mg, 0.023 mmol) in a v 5 mL v vial equipped with spin bar were added fresh toluene (2 mL) and *n*-butanol (4 mL). Ethylenediamine was added and the reaction mixture was heated at 90 o C for 18 hr. After cooling to room temperature, the reaction mixture was concentrated under *vacuo*. The crude product was dissolved in 5 mL of toluene and evaporated to dryness. Pyridine and acetic anhydride were added and the reaction mixture was stirred for 16 hr at room temperature. The reaction mixture was evaporated to dryness, twice from toluene, yielding foam with some solid.

This material was dissolved in 5 mL of methanol and 2 mL of THF under argon and 0.35 mL of 25% sodium methoxide in methanol was added and the reaction mixture was stirred for 12 hr. Solid ammonium chloride was added and stirred for 30 min. Careful evaporation of this biphasic reaction mixture provided white solid residue, which was washed three times by ethyl acetate. Concentration of ethyl acetate layer yielded the crude product, which was purified by preparative TLC (10% ethanol in toluene) to afford **28** in 69% yield (0.064 gm). R_f = 0.67 (10 ethanol in toluene). [α] + 14.6 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.65 (2H, d, J = 9 Hz), 7.60 (2H, d, J = 7.5 Hz), 5.22 (1H, d, J = 8 Hz), 5.10 (1H, br-s), 5.07 (2H, br-s), 3.06 (1H, m), 2.96 (1H, m), 2.24 (2H, d, J = 14.5 Hz), 1.68 (3H, s), 0.90 (9H, s), 0.07 (3H, s), 0.027 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz).□ 169.6, 141.5, 140.9, 139.6, 139.3, 139.1, 138.97, 138.95, 138.89, 138.7, 138.6, 138.5, 138.39, 138.36, 138.3, 138.2, 138.0, 137.7, 132.6, 132.4, 129.0, 128.9, (128.7-127.3), 127.2, 126.7, 103.0, 102.0, 100.9, 100.0, 99.9, 98.3, 97.793.0, 82.5, 81.4, 80.2, 80.1, 79.7, 79.4, 78.8, 78.6, 78.1, 77.9, 77.8, 76.6, 76.0, 75.3, 75.2, 74.9, 74.8, 74.7, 74.4, 74.38, 74.35, 74.2, 73.9, 73.7, 73.6, 73.5, 73.49, 73.45, 73.37, 73.2, 73.1, 72.0, 71.9, 71.8, 71.4, 71.4, 71.3, 71.1, 70.0, 69.7, 69.1, 68.9, 68.7, 68.4, 67.9, 67.8, 66.7, 65.7, 58.8, 58.2, 57.2, 26.0, 23.6, 18.2, 1.2, -4.2, 5.4.



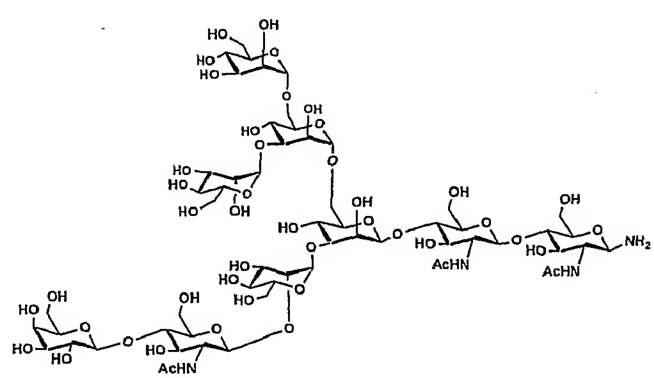
29

To the azeotropically dried **28** in a 25 mL round bottomed flask equipped with stir bar was added 0.5 mL 1M acetic acid in THF and the reaction mixture was cooled to 0 °C. To this ice cooled reaction mixture was added 0.5 mL TBAF (1M in THF). The cooling bath was removed and the reaction mixture was stirred for 3 hr. Additional 2 mL 1M acetic acid in THF was added and the reaction mixture was stirred for 15 min. The reaction mixture was evaporated to dryness and the crude product was purified by preparative TLC (10% ethanol in toluene) to afford 0.055 gm (89% yield)of **29**. [α] + 9.40 (c 1, $CHCl_3$); ^1H - NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.66 (2H, d, J = 8 Hz), 7.60 (2H, d, J = 7.6 Hz), 5.17 (1H, d, J = 7.2 Hz), 5.07 (1H, br-s), 5.01 (2H, m), 2.34 (1H, br), 2.18 (1H, br), 1.61 (s, 3H),



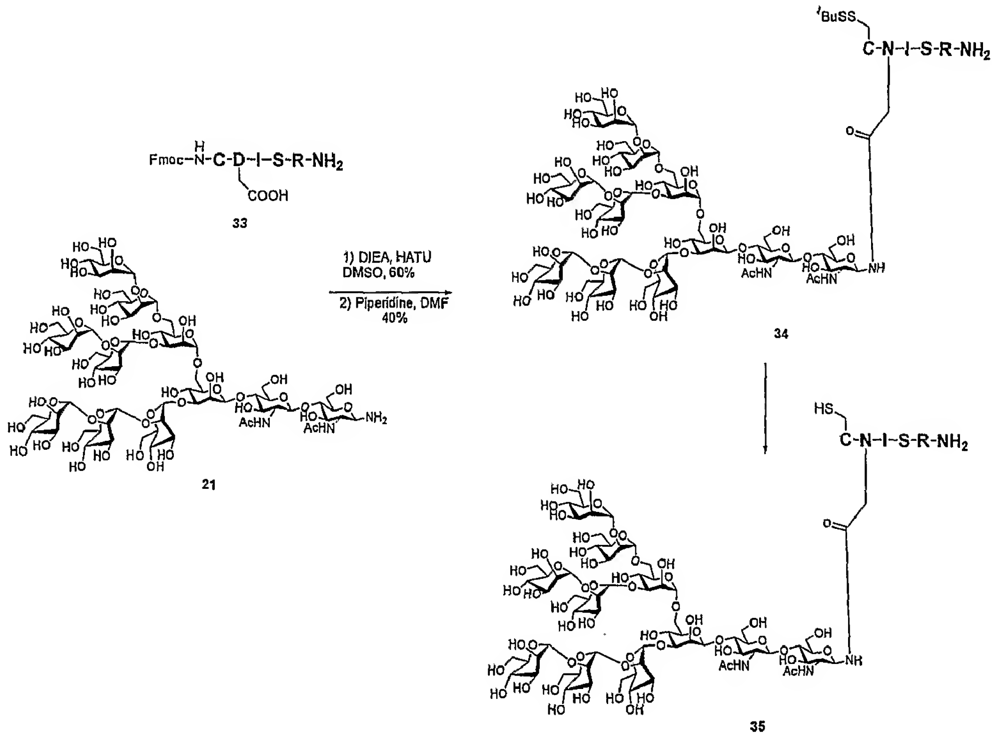
30

Into a three necked round bottomed flask, equipped with dry ice acetone condenser was condensed 15 mL ammonia under argon. Sodium metal (0.095 gm, 153 equiv.) was added in three portions. The resulting blue solution was stirred for 30 min at − 78 °C. The compound **29** (0.104 gm, 0.027 mmol) in 1.5 mL (3 x 0.5 mL) was added to the solution and the reaction mixture was stirred for 2 hr. Solid ammonium chloride (0.263 gm, 4.97 mmol) was added to quench the reaction and the reaction mixture was warmed to room temperature slowly. Evaporation of the residual liquid provided solid residue, which was dissolved in 5 mL pyridine. To this mixture was added acetic anhydride (3 mL) and DMAP (5 mg) and the resulting mixture was stirred with slowly warming to room temperature over 12 hr. The reaction mixture was evaporated to dryness and purified carefully by silica gel column chromatography to afford peracetate. The peracetate in 5 mL methanol was added solution of NaOMe, 25% by weight in methanol (0.4 mL) and was stirred for 24 hr. The resulting cloudy solution was treated with water at 0 °C and stirred for another 24 hr. The reaction mixture was neutralized using Amberlyst − 15 acidic regin and evaporated to afford crude product, which was purified by size exclusion chromatography using Bio-Gel P2 regin yielding 30 mg of free glycan.



31

Free glycan (10 mg) in 15 mL of saturated ammonium bicarbonate was heated at 40 °C. Additional ammoniumhydrogen carbonate was added time to time to keep the solution saturated. After two days of stirring the content of the flask was shell frozen, lyophilized, dissolved in water (10 mL), lyophilized; this process was repeated until the white solid residue reached constant mass of 10 mg, which was used directly in the next step.



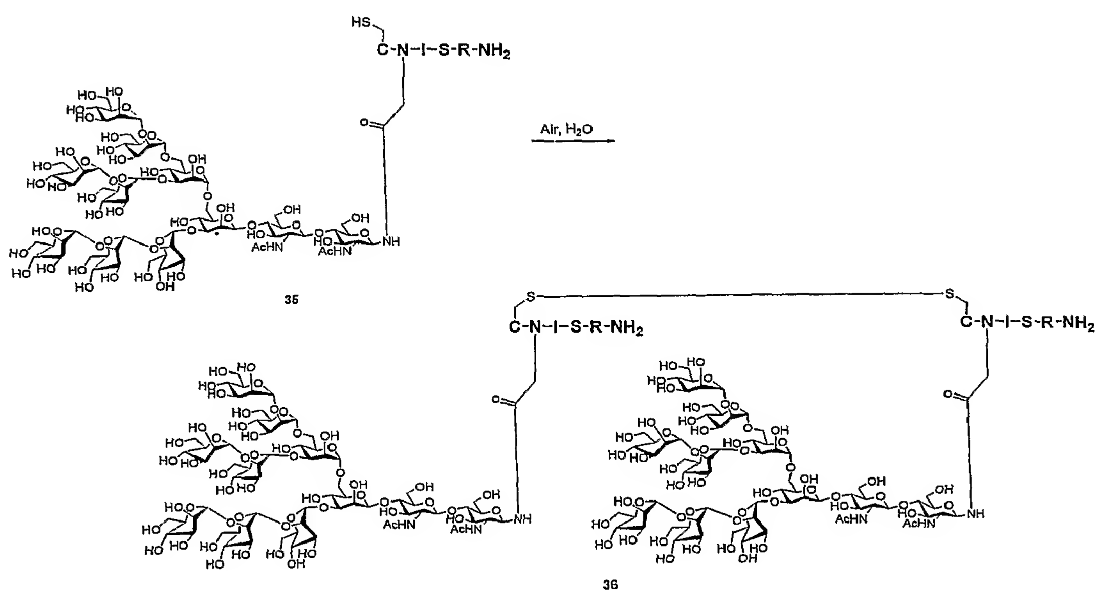
Glycopeptide 34:

A solution of acid **33** (6 mg, 0.007 mmol), HATU (5 mg, 0.013 mmol), DIEPA (1.7 µL, 0.012 mmol) in DMSO (0.1 mL) was stirred for 10 min and transferred to a falcon tube (25 mL) containing 4.2 mg of **21**. The solution was stirred for 2 h and additional DIEPA (1.2 µL) was added. The reaction mixture was purified by semiprep HPLC column (30 to 50%B over 20 min) to afford Fmoc-protected glycopeptide (3.6 mg, 60%). LRMS (ESI) calcd for

$C_{111}H_{177}N_{12}O_{64}S_2Na^{++}$ $[M+H+Na]^{++}$ 1394.5, found 1394.5. This Fmoc-protected glycopeptide was dissolved in 0.4 mL pipyridine/DMF (1:4) solution and stirred for 15 min and quenched by THF/H_2O (10%) until the pH = 2~3. The crude mixture was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **34** (2 mg, 40%). LRMS (ESI) calcd for $C_{96}H_{167}N_{12}O_{62}S_2Na^{++}$ $[M+H+Na]^{++}$ 1283.5, found 1283.6. ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.02 (s, 1 H), 5.16 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).

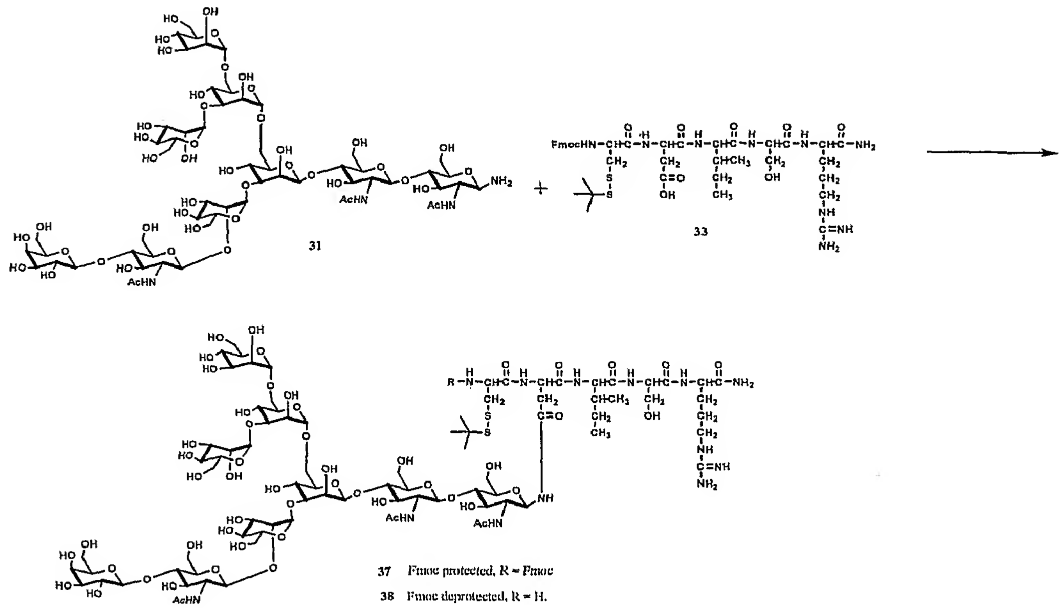
Glycopeptide 35:

To a solution of **34** (2 mg, 0.0008 mmol) in phosphorous buffer (NaH_2PO_4 and Na_2HPO_4, pH=7.4, 0.5 mL) was added $HSCH_2CH_2SO_3Na$ (10 mg, 0.061 mmol) and stirred for 2 days. TCEP (30 mg, 0.104 mmol) was then added and the resulting solution was stirred for 1 h. The residue was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **35** (1.7 mg, 60%). LRMS (ESI) calcd for $C_{92}H_{160}N_{12}O_{62}S^{++}$ $[M+2H]^{++}$ 1228.5, found 1228.5. ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.90 (s, 1 H), 4.99 (s, 1 H), 5.15 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).



Glycopeptide, when dissolved in H_2O and exposed to air, formed

dimmer **36**. HPLC (Varian Microsorb 100-5-C18) retention time, 12.75 min (0% to 40% acetonitrile in water). LRMS: (ESI) $C_{184}H_{317}N_{24}O_{124}S_2$: $[M+3H]^{+++}$: calculated: 1636.95, found, 1636.99.

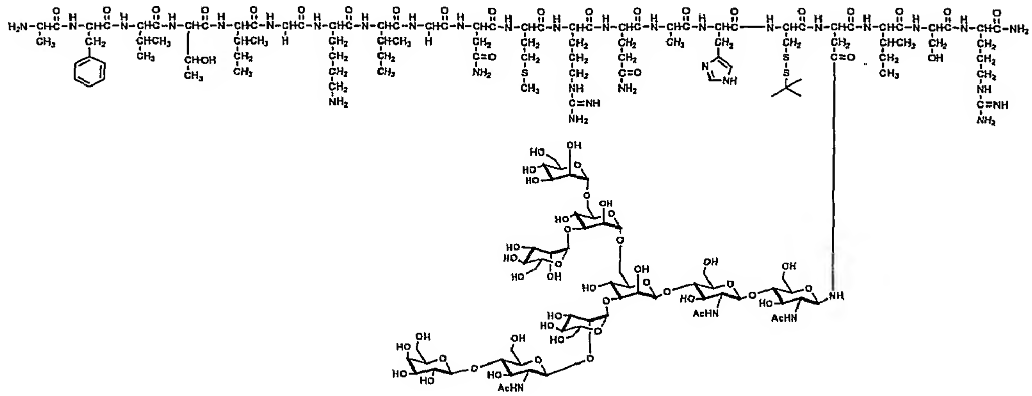


37 Fmoc protected, R = Fmoc

38 Fmoc deprotected, R = H.

To a 15 mL polypropylene conical tube equipped with stir bar in 0.2 mL DMSO was added peptide **33** (11 mg, 3 equiv.) and HATU (15 mg, 5.9 equiv.). The solution was stirred for 1 min and was added diisopropyl ethyl amine (3.58 µL, 3 equiv.) and was stirred for another minute. This orange-yellow solution was transferred *via* 0.5 mL syringe to the flask containing glycosylamine **31** (11 mg). The conical tube was rinsed with additional 0.1 mL of DMSO and transferred to the flask containing glycosyl amine using the same 0.5 mL syringe. Monitoring by LCMS showed that no additional product formation after 6 hr. Purification of the reaction mixture by size exclusion chromatography provided the **37**. To this Fmoc protected **38** was added a 1 : 3 : 16 mixture of hydrazine : piperidine : DMF (200 µL). The resulting yellowish solution was stirred for 30 min before addition of a solution of TFA to bring the pH to 3. The reaction mixture was purified by semiprep HPLC column (5 to 25%B over 25 min) to afford the

Fmoc deprotected **38** in 30% yield. ^1H - NMR ($CDCl_3$, 500 MHz) (selected protons) δ 4.78 (2H, d, J = 12.4 Hz), 4.70 (1H, d, J = 9.6 Hz), 4.57 (2H, d, J = 13.2 Hz), 4.26 (4H, m), 4.14 (1H, d, J = 7.2 Hz), 1.01 (9H, s), 0.575 (6H, m).



39

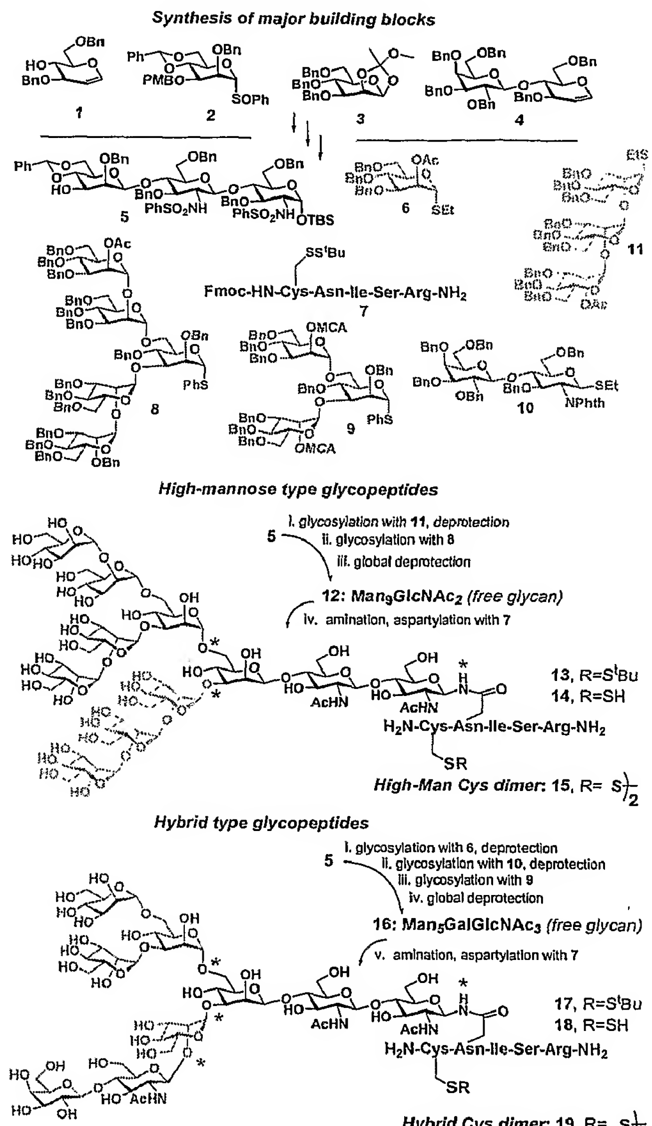
Compound **39** was prepared similar to **34**.

Experimental Details Part II

The study commenced with the preparation of the major oligosaccharide buiding blocks including the core beta-mannose/chitobiose trisaccharide **5**. The "D1 arm" saccharides **10** and **11** of the high mannose and hybrid glycans respectively, and the upper domains, i.e. pentasaccharide **8** and trisaccharide **9** branches were also synthesized (Scheme 11). These fragments were appropriately assembled to provide free **12** and **16**. The reducing termini of these fully synthetic oligosaccharides were then aminated as previously described, building on earlier protocols of Kochetkov and Lansbury Each glycosylamine was coupled to a Cys - protected $gp120^{331-335}$ pentapeptide.

Scheme 14

Synthesis of gp120$^{331-335}$ glycopeptides carrying high-mannose and hybrid-type fragments



The resulting Cys-blocked glycopeptides **13** and **17** were reduced to liberate the Cys sulfhydryl function, thereby affording compounds **14** and **18,** respectively. Described herein is the first real-time analyses of 2G12 binding to these gp120 targeted constructs.

Binding analyses utilized the Surface Plasmon Resonance (SPR) technology, and were carried out using the Biacore 3000 system (See **Figure 1a** and Table 1). 2G12 and a human IgG1 isotype-control antibody were immobilized by the amine coupling method to a CM5 sensor chip, generating the active and reference surfaces. A single injection of the tested material resulted in its successive exposure first to the reference surface, and then to the active surface. Each binding profile represents an automatic subtraction of the reference surface signal from the 2G12 surface signal. Binding experiments were performed at 25 °C in HBS-P buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005 % Surfactant P20). The sensor surface was regenerated with a short pulse of 3.5 M $MgCl_2$. Recombinant HIV-1_{JR-FL} gp120 was tested for comparison.

With the synthetic gp120 glycopeptides in hand, we could probe their binding to 2G12. In the high-mannose series, free glycan **12** binding was below detection threshold, however glycan/pentapeptide conjugate with free Cys SH **14** demonstrated significant binding with 2G12. At the same time, the conjugate with the protected sulfhydryl function (**13**) showed only very low level of binding (Table 1). The high sensitivity of binding of 2G12 to the apparent state of the sulfur atom in the N-terminal cysteine was initially puzzling, given the perception that binding is in either case directed to the glycan domain.

Table 1. Qualitative assessment of 2G12 binding.

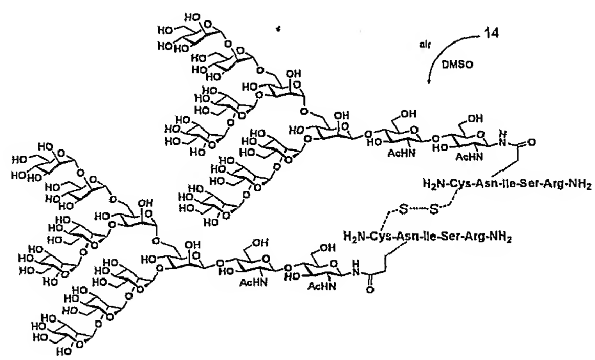
Compound (concentration)	*Carbohydrate type*	*Cys SH state*	*Binding, RU*
12 (40 µM)	High-mannose	none	<1
13 (20 µM)	High-mannose	blocked	5
14 (10 µM)	High-mannose	free	75
14 (10 µM)+DTT	High-mannose	free	9.5
15 (10 µM)	High-Man dimer	dimer	78
16 (40 µM)	Hybrid	none	<1
17 (20 µM)	Hybrid	blocked	<1
18 (20 µM)	Hybrid	free	<1
19 (20 µM)	Hybrid dimer	dimer	<1

An important clue arose upon examination of the H_2O stock solution of the presumed thiol **14**. Liquid chromatography /mass spectroscopy (LCMS) analysis indicated that this material was now actually a mixture of the monomeric and the oxidized disulfide forms, with a prevalence of the latter. Moreover, treatment of the compound **14** stock (0.5 mM as per compound monomer) with dithiotreitol (DTT) at 25 mM (50 fold molar excess as calculated per compound monomer) resulted in significantly reduced binding (**Figure 1b**).

In control experiments, it was confirmed that after the passage of the DTT-pretreated sample, the 2G12 surface retains the ability to bind the unreduced compound. In another control, we similarly used 25 mM DTT to pretreat the gp120 stock (5 µM), and detected no significant effects on 2G12 binding despite an even greater (5,000-fold) excess of DTT over gp120.

These experiments, in the aggregate, suggested that the dimeric form of the glycopeptide is responsible for observed 2G12 binding. Indeed, when dimer **15** was then prepared, in homogeneous form, by DMSO oxidation of **14,** it exhibited strong binding to 2G12 (**Figure 1a**).

Formation of the high-mannose dimer **15**:



It was then evaluated the corresponding set of gp120 constructs, but now carrying hybrid type carbohydrates, which lack the lower trimannose (D1) arm present in the high - mannose glycans. In hybrid compounds **16 - 19** this sector is replaced by an *N*-acetyllactosamine residue (**Figure 1a**). Additionally, the upper pentasaccharide branch in hybrids **16 - 19** is trimmed to the trisaccharide level. *It was found that none of the constructs possessing the hybrid-type glycan pattern, including the dimeric structure (see* ***19****) showed any detectable binding*. While glycosidase digestion studies have identified such hybrid elements in gp120, our findings demonstrate that it is not recognized by 2G12. The sensitivity of binding to the multimeric character of the glycan is certainly in keeping with the structural notions offered by Wilson.

To probe whether the dimeric high mannose compound **15** and gp120 recognize the same site on 2G12, competition binding

experiments were performed (**Figure 2**). Compound **15** was injected into the flow cell at concentrations up to 10 μM, followed by an injection of gp120 at a constant concentration of 12.5 nM. Increasing the amount of pre-bound **15** resulted in progressive inhibition of gp120 binding. The monomeric form of the glycopeptide **14** did not block gp120 binding, as expected. In reciprocal experiments, pre-bound gp120 (0-100 nM) also progressively inhibited the binding of the compound **15** (2.5 μM) to 2G12. These results indicate that gp120 and glycopeptide **15** compete for binding to 2G12, *supporting the idea that the dimeric glycopeptide binds to 2G12 by mimicking the clustered gp120 epitope.*

The observed binding profile of dimer **15** points to a rather complex dynamics (**Figure 3**) that does not fit into a simple 1:1 Langmuir model. The observed profile can be viewed as including two association (fast and slow) and two dissociation (fast and slow) components, and may indicate ~~a~~ required conformational adjustment for the binding of the second glycan. Our finding that the clustered construct demonstrates significantly stronger binding than the monomeric glycopeptide is in agreement with the co-crystal structure of the 2G12/high-mannose sugar complex, where at least two polysaccharides bind to spacially adjacent pockets on the surface of the antibody. Further optimization of the linker between the polysaccharides is a promising direction for design of antigens intended for use in HIV vaccines.

The constructs described above will be evaluated as part of our HIV vaccine quest. At the same time, designs of later generation vaccine candidates are moving forward. These ongoing investigations build upon the key observations described above and are enabled by the major advances in the

synthesis of complex glycopeptides.

Characterization of the binding of the DG-I-103, DG-1-107, MM1-165 compounds to the 2G12 antibody

The goal of the study was to investigate the ability of DG-I-103 (high-mannose type glycan attached to the gp120$^{331-335}$ pentapeptide), DG-I-107 (high-mannose type glycan), and MM1-165 (hybrid-type glycan) to bind to 2G12 and to characterize the specificity and affinity of the binding.

Surface plasmon resonance analyses were performed using a Biacore 3000 system. 2G12 and HuIgG1 were immobilized at 10,000 resonance units by the amine coupling method to a CM5 sensor chip, according to the manufacturer's instructions. Binding experiments were performed at 25^{0}C in HBS-P buffer (10mM HEPES pH7.4, 150 mM NaCl, 0.005% Surfactant P20) at a flow rate of 10 l/min. The sensor surface was regenerated with a short pulse of 3.5M $MgCl_2$.

Both gp120 and the gp120 glycopeptide appeared to specifically bind 2G12 in these preliminary experiments (**Figure 4**). The maximum level of binding was ~800 resonance units (RU) for gp120 and ~20 RU for the gp120 glycopeptide. The difference in maximum RU values is in good agreement with the difference in the molecular weights of the molecules (~92,000 Da for gp120 and 2456 Da for the gp120 glycopeptide). The result is noteworthy given that RU values are directly proportional to the mass of the bound ligand.

In these experiments, the gp120 glycopeptide was used at a 100X higher concentration than gp120 (10 μM v. 0.1 μM), suggesting a difference in affinity for 2G12. However,

additional experiments are required to accurately assess the relative affinities of the two compounds for 2G12, particularly given the slow off-rates observed for both molecules.

No specific binding to 2G12 was observed for either the gp120 glycan or the unrelated glycan (**Figure 4**). The findings are intriguing in suggesting that peptidic elements influence 2G12 binding.

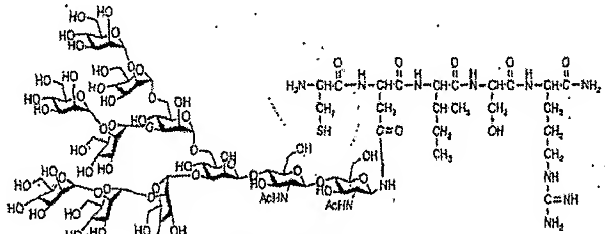
Characterization of the binding of the DG-I-103, DG-1-152B, MM1-204, DV-C53, and DG-I-107 compounds to the 2G12 antibody

Surface plasmon resonance analyses were performed using a Biacore 3000 system. 2G12 and HuIgG1 were immobilized at 10,000 resonance units (RU) by the amine coupling method to a CM5 sensor chip, according to the manufacturer's instructions. Binding experiments were performed at 25^0C in HBS-P buffer (10mM HEPES pH7.4, 150 mM NaCl, 0.005% Surfactant P20) at a flow rate of 10 ul/min. The sensor surface was regenerated with a short pulse of 3.5M $MgCl_2$.

All compounds were reconstituted in thoroughly degassed dH_2O, aliquotted (50 ul/aliquot) and stored at -80°C.

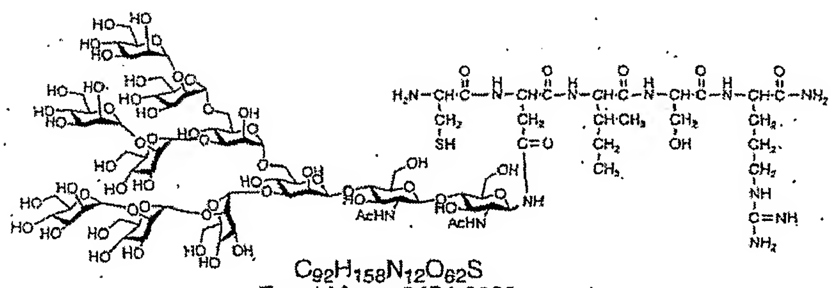
DG-I-103 (high-mannose type glycan attached to the gp120[331-335] pentapeptide) MW: 2,456

Lot#1: 0.1 mg reconstituted in 400 ul dH_2O; 0.1mM stock

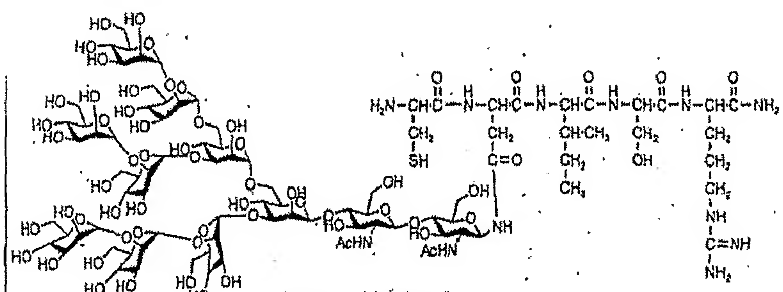


$C_{92}H_{158}N_{12}O_{62}S$
Exact Mass: 2454.9300
Mol. Wt.: 2456.3471
DG-I-103, 0.1 mg

Lot#2: 0.1mg+ Lot#3: 0.3mg reconstituted in 320ul dH_2O; 0.5mM stock



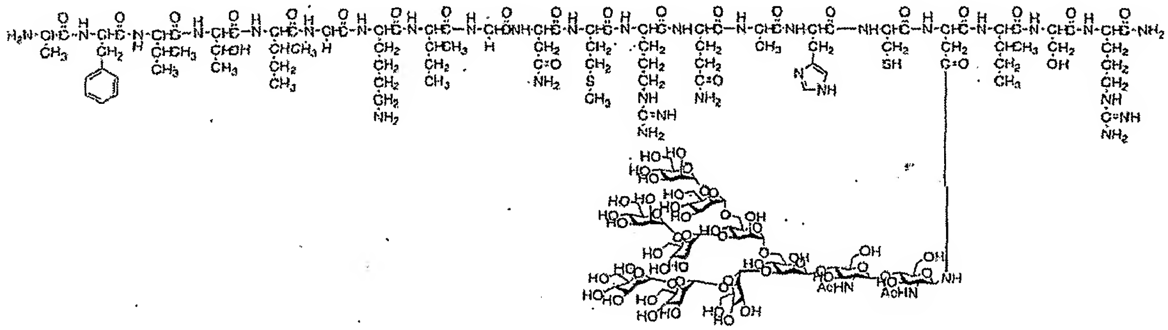
$C_{92}H_{158}N_{12}O_{62}S$
Exact Mass: 2454.9300
Mol. Wt.: 2456.3471
DG-I-103, 0.1 mg



$C_{92}H_{158}N_{12}O_{62}S$
Exact Mass: 2454.9300
Mol. Wt.: 2456.3471
DG-I-103, 0.3 mg

DG-I-152B (high-mannose type glycan attached to the gp120$^{316-335}$ 20mer-peptide) MW: 4,081

Lot#1: 0.5mg reconstituted in 240ul dH_2O; 0.5mM stock

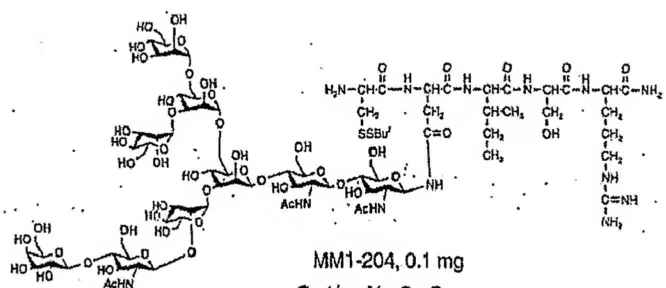


DG-I-152B, 0.5 mg

$C_{164}H_{275}N_{35}O_{80}S_2$
Exact Mass: 4078.7968
Mol. Wt.: 4081.2548

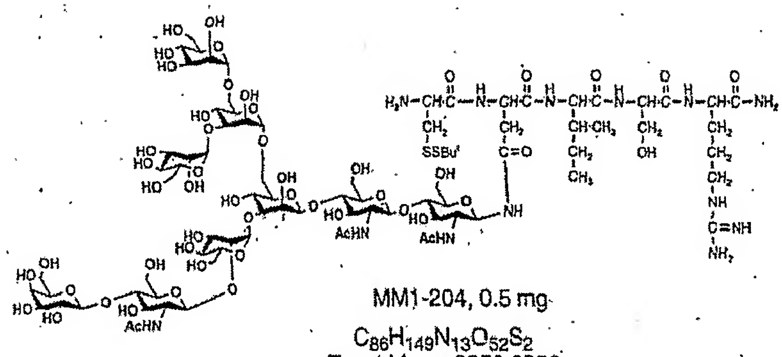
MM-I-204 (hybrid-mannose type glycan attached to the gp120$^{331-335}$ pentapeptide) MW: 2,261

Lot#1: 0.1mg+ Lot#2: 0.5mg reconstituted in 265ul dH_2O; 1mM stock



MM1-204, 0.1 mg

$C_{86}H_{149}N_{13}O_{52}S_2$
Exact Mass: 2259.8856
Mol. Wt.: 2261.2892

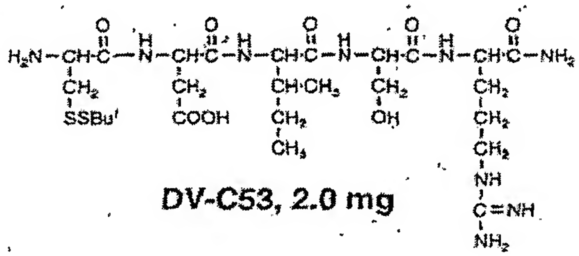


MM1-204, 0.5 mg

$C_{86}H_{149}N_{13}O_{52}S_2$
Exact Mass: 2259.8856
Mol. Wt.: 2261.2892

DV-C53 (gp120$^{331-335}$ pentapeptide) MW: 558

Lot#1: 2mg reconstituted in 358ul dH_2O; 10mM stock

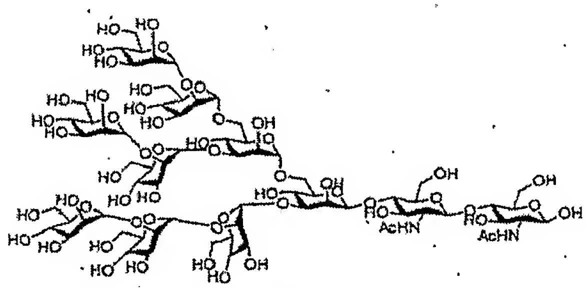


DV-C53, 2.0 mg

$C_{22}H_{40}N_9O_8$
Exact Mass: 558.3000
Mol. Wt.: 558.6085

DG-I-107 (high-mannose type glycan) MW: 1,884

Lot#2: 0.7mg reconstituted in 186ul dH_2O; 2mM stock



DG-I-107, 0.7 mg

$C_{70}H_{118}N_2O_{56}$
Exact Mass: 1882.6447
Mol. Wt.: 1883.6657

DG-I-103 and DG-I-152B specifically bind 2G12 with different affinities and binding profiles (**Figure 5a**). Interestingly, the new batch of DG-I-103 (lot#2+lot#3 received on 6/11/03 and 8/08/03; combined and reconstituted on 8/28/03) binds 2G12 much better than the previous batch (lot#1; received on 3/27/03; reconstituted on 4/17/03) (140 RU as compared to 21 RU, data not shown).

Storage (at −80°C) and/or freezing-thawing of DG-I-103 results in reduced binding to 2G12, and in changes in the binding profile (**Figure 5b**). This effect could be related to a change in the structure of the compound, such as dimerization via S-S bonds between Cys residues. This possibility is supported by Vadim's LCMS analysis (personal communication), which indicated that a frozen aliquot represented a mixture of monomeric and dimeric forms, with a prevalence of the dimeric form. The addition of DTT (25 mM, i.e. 50 fold molar excess relative to the compound) led to a full conversion to the monomeric form.

To test whether such dimerization is responsible for the observed reduction in binding, a frozen aliquot of the compound was treated with a range of DTT concentrations (0-25 mM) for 1 hr at RT. The treated samples were diluted 50 fold, and tested for binding with 2G12 (**Figure 5c**). DTT at concentrations 5- and 50-fold higher than that of the compound resulted in significantly reduced binding, whereas DTT at a concentration 200-fold below that of the compound had little or no effect on binding. At the end of the series, a control run with an untreated sample confirmed that the 2G12 surface remained intact.

Treatment of gp120 with DTT (25 mM, 5000-fold molar excess) resulted in a modest decrease in binding. The finding indicates that DTT does not exert a large transient effect on 2G12. As before, the 2G12 surface remained intact following regeneration (**Figure 5d**).

Comparison of binding profiles for several compounds suggests that high-mannose type glycan attached to the peptides is essential for binding to 2G12 (**Figure 6**). In light of the above DTT experiments, however, it remains possible that the differential binding of these compounds results from the different state of the Cys residue (blocked/unblocked) which affects the oligomeric state of the compound. Thus, we cannot exclude the possibility that 2G12 may bind dimeric forms of the peptides either free or linked to the mixed-type glycan. Further experiments are needed which will use compounds with uniformly unblocked (or uniformly blocked) Cys residues and defined oligomeric states.

Preliminary experiments support the notion that compound A (**DG-I-103**) and gp120 compete for 2G12 binding. Compound A shows reduced binding to a 2G12 surface if this surface has been pre-blocked by gp120 (**Figure 7a**). In a reciprocal experiment, gp120 appears to show reduced binding to a 2G12 surface pre-blocked by compound A (**Figure 7b**). These results provide preliminary support to the idea of competitive binding. It should be noted that blocking the 2G12 surface by gp120 (**Figure 7a**) or compound A (**Figure 7b**) did not reach saturation, thus the competition was not complete.

The state of the peptide cysteine residue (*i.e.*, blocked, oxidized, reduced) profoundly affects binding of gp120 glycopeptides to 2G12. The data are consistent with a model where high-level binding is observed exclusively for multimeric glycopeptides. Notably, the crystal structure of 2G12 revealed a total of four $Man_9GlcNAc_2$ moieties bound to each Fab 2G12 dimer (Calarese et al., Science, 300:2065, 2003).

References

1. Burton, D. R. *Proc. Natl. Acad. Sci. U S A* **1997**, *94*, 10018-10023.

2. Wei, X. P.; Decker, J. M.; Wang, S. Y.; Hui, H. X.; Kappes, J. C.; Wu, X. Y.; Salazar-Gonzalez, J. F.; Salazar, M. G.; Kilby, J. M.; Saag, M. S.; Komarova, N. L.; Nowak, M. A.; Hahn, B. H.; Kwong, P. D.; Shaw, G. M. *Nature* **2003**, *422*, 307-312.

3. Wyatt, R.; Sodroski, J. *Science* **1998**, *280*, 1884-1888.

4. Leonard, C. K.; Spellman, M. W.; Riddle, L.; Harris, R. J.; Thomas, J. N.; Gregory, T. J. *J. Biol. Chem.* **1990**, *265*, 10373-10382.

5. Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science* **2003**, *300*, 2065-2071.

6. Trkola, A.; Purtscher, M.; Muster, T.; Ballaun, C.; Buchacher, A.; Sullivan, N.; Srinivasan, K.; Sodroski, J.; Moore, J. P.; Katinger, H. *J. Virol.* **1996**, *70*, 1100-1108.

7. Baba, T. W.; Liska, V.; Hofmann-Lehmann, R.; Vlasak, J.; Xu, W. D.; Ayehunie, S.; Cavacini, L. A.; Posner, M. R.; Katinger, H.; Stiegler, G.; Bernacky, B. J.; Rizvi, T. A.; Schmidt, R.; Hill, L. R.; Keeling, M. E.; Lu, Y. C.;

Wright, J. E.; Chou, T. C.; Ruprecht, R. M. *Nat. Med.* **2000**, *6*, 200-206.

8. Scanlan, C. N.; Pantophlet, R.; Wormald, M. R.; Saphire, E. O.; Stanfield, R.; Wilson, I. A.; Katinger, H.; Dwek, R. A.; Rudd, P. M.; Burton, D. R. *J. Virol.* **2002**, *76*, 7306-7321.

9. Sanders, R. W.; Venturi, M.; Schiffner, L.; Kalyanaraman, R.; Katinger, H.; Lloyd, K. O.; Kwong, P. D.; Moore, J. P. *J. Virol.* **2002**, *76*, 7293-7305.

10. Lee, H. K.; Scanlan, C. N.; Huang, C. Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 1000-1003.

11. Wang, L. X.; Ni, J. H.; Singh, S.; Li, H. G. *Chem. Biol.* **2004**, *11*, 127-134.

12. Miller, J. S.; Dudkin, V. Y.; Lyon, G. J.; Muir, T. W.; Danishefsky, S. J. *Angew Chem Int Edit* **2003**, *42*, 431-+.

13. Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 736-738.

14. Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. *Tetrahedron. Lett.* **2003**, *44*, 1791-1793.

15. Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1-C5.

16. Cohen-Anisfeld, S. T.; Lansbury, P. T. *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537.

17. Mandal, M.; Dudkin, V. Y.; Geng, X.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2004**, in press.

18. Geng, X.; Dudkin, V. Y.; Mandal, M.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2004**, in press.

19. Rich, R. L.; Myszka, D. G. *Curr. Opin. Biotech.* **2000**, *11*, 54-61.

20. Rich, R. L.; Day, Y. S. N.; Morton, T. A.; Myszka, D. G. *Anal. Biochem.* **2001**, *296*, 197-207.

21. Trkola, A.; Dragic, T.; Arthos, J.; Binley, J. M.; Olson, W. C.; Allaway, G. P.; Cheng-Mayer, C.; Robinson, J.; Maddon, P. J.; Moore, J. P. *Nature* **1996**, *384*, 184-187.

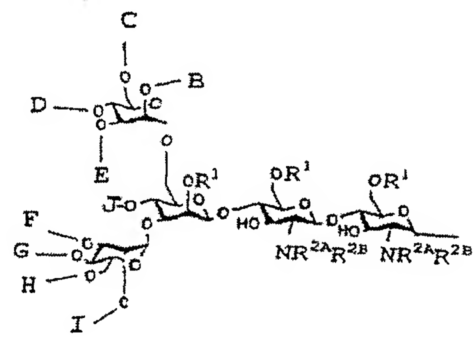
22. Rich, R. L.; Myszka, D. G. *Trends Microbiol.* **2003**, *11*, 124-133.

What is claimed is:

1. A compound represented by the formula:

(A~X---)$_n$

wherein each A~ is independently a carbohydrate represented by the general structure:



wherein each of B, C, D, E, F, G, H, I, and J independently represents a sugar moiety, a sugar moiety having a protecting group attached thereto, a hydrogen, or an oxygen protecting group, provided that no more than two of B, C, D, and E is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and that no more than one of F, G, H, and I is simultaneously a sugar moiety or a sugar moiety having a protecting group attached thereto and wherein ~ represents a covalent bond between the carbohydrate and the peptide;

wherein each each R^1 independently represents a hydrogen or an oxygen protecting group;

wherein each R^{2A} and each R^{2B} independently represents a hydrogen or a nitrogen protecting group;

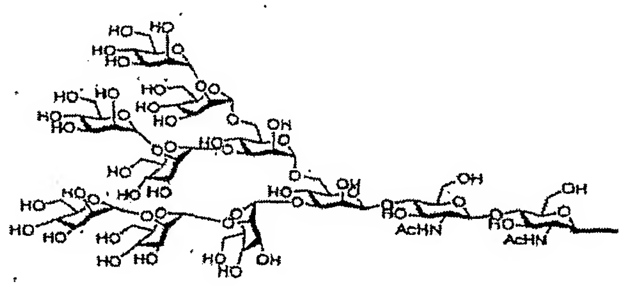
wherein X is a peptide comprising 5-20 amino acids, at

least one amino acid being a cysteine residue and at least one other amino acid being an asparagine or a glutamine residue;

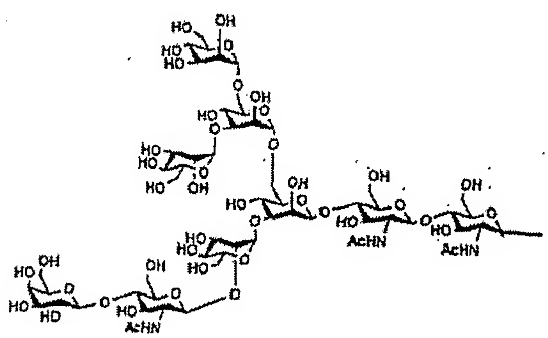
wherein --- represents a disulfide bond between the sulfur of a cysteine in one peptide and the sulfur of another cysteine in another peptide; and

wherein n represents an integer ≥ 2.

2. The compound of claim 1, wherein each A~ is independently a carbohydrate having one of the following structures:



; or



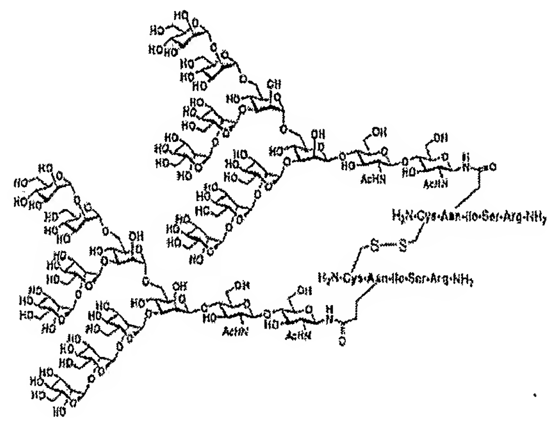
3. The compound of claim 1, wherein the peptide comprises consecutive amino acids, the sequence of which is present in gp120 of HIV virus.

4. The compound of claim 1, wherein ~ represents a covalent

bond between a carbohydrate and an amide moiety of an asparagines or a glutamine residue present on the peptide.

5. The compound of claim 1, wherein n equals 2.

6. The compound of claim 5 having the structure:

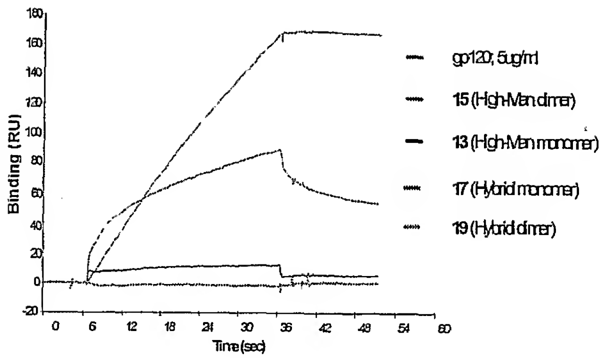


7. A composition comprising the compound of any of claims 1-6 and a carrier.

8. The composition of claim 7 further comprising an immune response stimulating amount of an immunostimulatory adjuvant.

9. A method of eliciting an immune response against HIV-1 or an HIV-1 infected cell in a subject which comprises administering to the subject an amount of the compound of any of claims 1-6 or a dose of the composition of claims 7 or 8 effective to elicit the immune response.

A


180
160
140
120
100
80
60
40
20
0
-20
Binding (RU)
0
6
12
18
24
30
36
42
48
54
60
Time (sec)
gp120; 5ug/ml
15 (High-Man dimer)
13 (High-Man monomer)
17 (Hybrid monomer)
19 (Hybrid dimer)

B

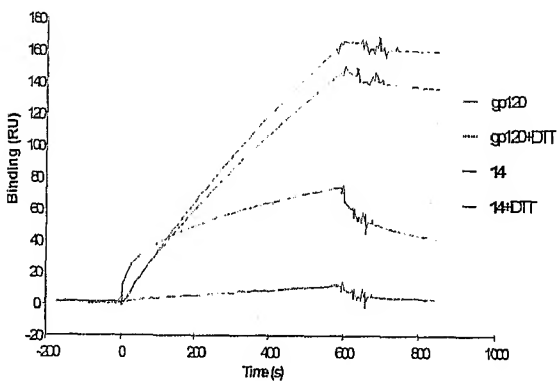
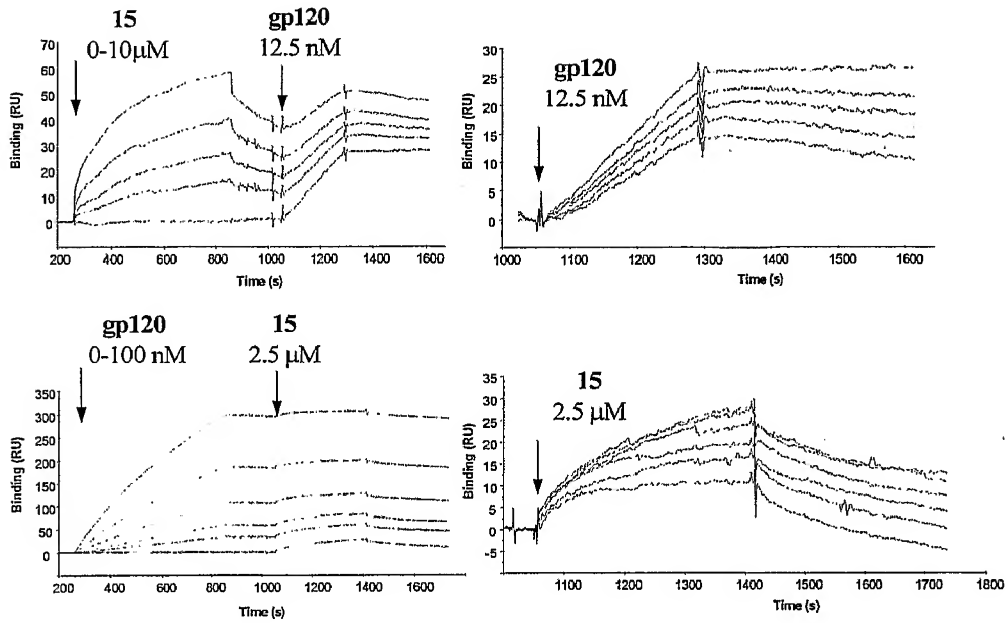

180
160
140
120
100
80
60
40
20
0
-20
Binding (RU)
-200
0
200
400
600
800
1000
Time (s)
gp120
gp120+DTT
14
14+DTT

Figure 1



15
0-10μM
gp120
12.5 nM
Binding (RU)
Time (s)
gp120
12.5 nM
Binding (RU)
Time (s)
gp120
0-100 nM
15
2.5 μM
Binding (RU)
Time (s)
15
2.5 μM
Binding (RU)
Time (s)

Figure 2

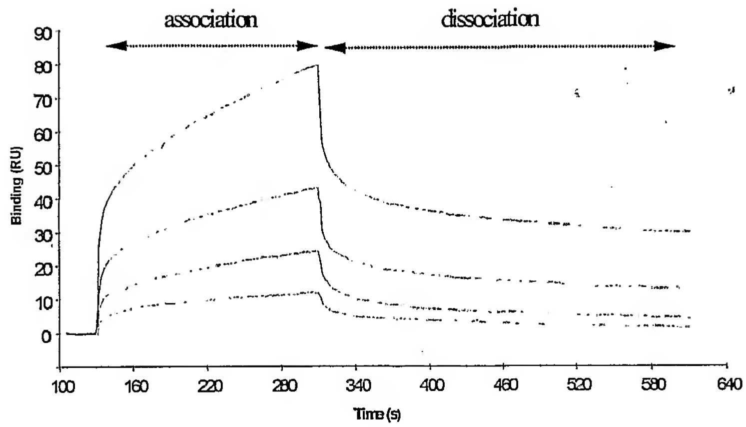
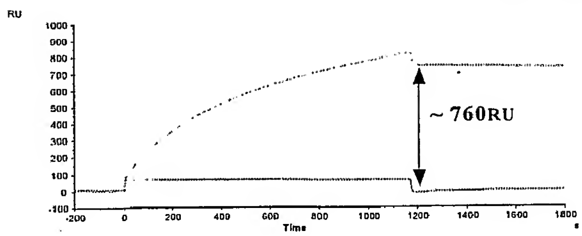
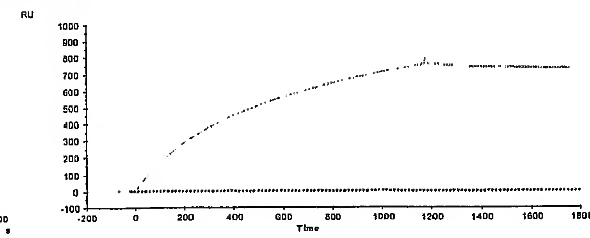

association
dissociation
Binding (RU)
90
80
70
60
50
40
30
20
10
0
100
160
220
280
340
400
460
520
580
640
Time (s)

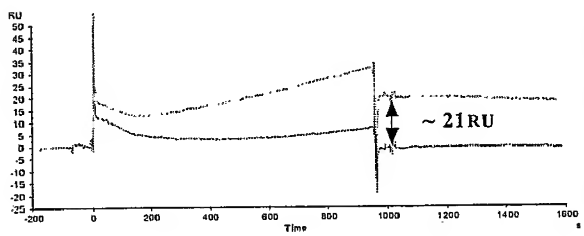
Figure 3

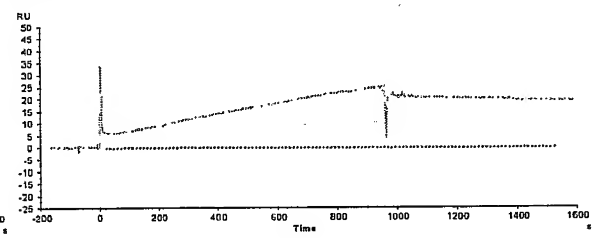
A **gp120, 0.1µM** B



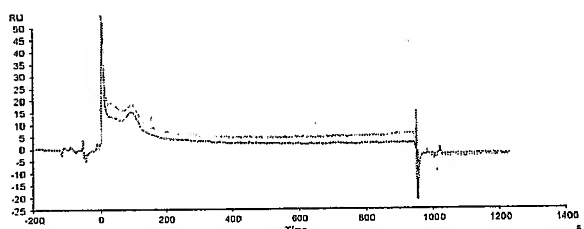


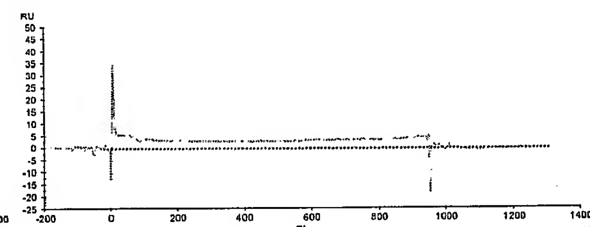
A **DG-I-103, 10µM** B



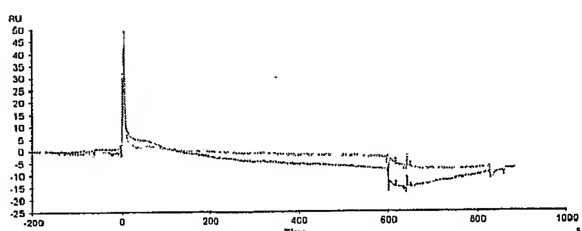


A **DG-I-107, 10µM** B





A **MM1-165, 10µM**



A. Representative sensograms with 2G12 (green) and HuIgG1 control (red) surfaces

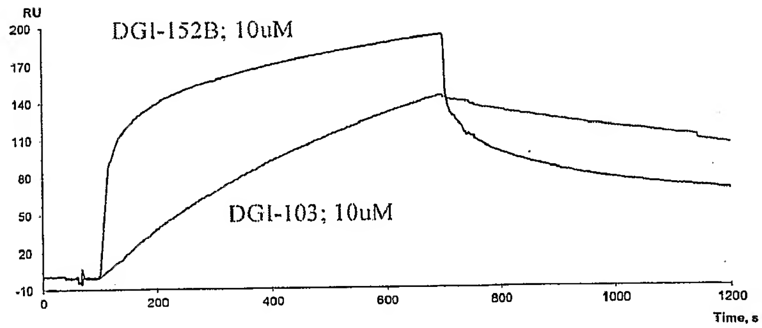
B. Net binding to 2G12, after subtraction of the background binding to HuIgG1.

2G12

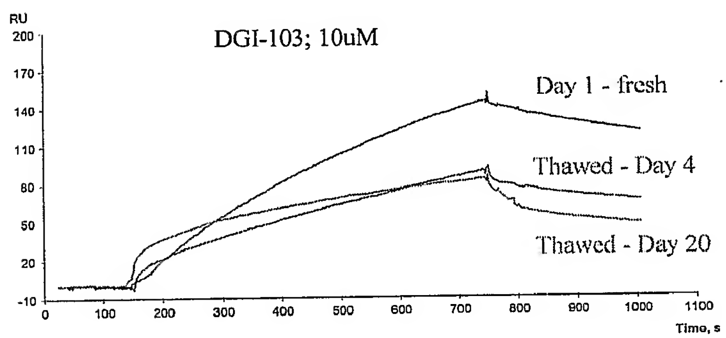
HuIgG1

Figure 4

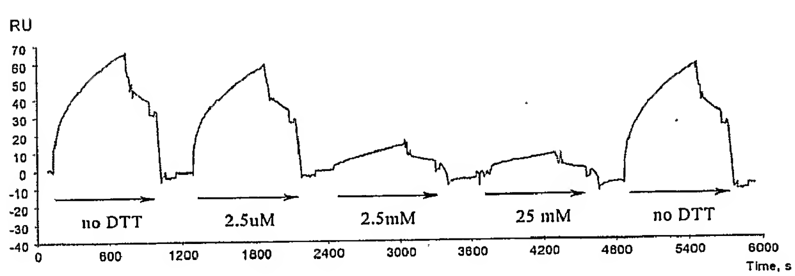
A



B



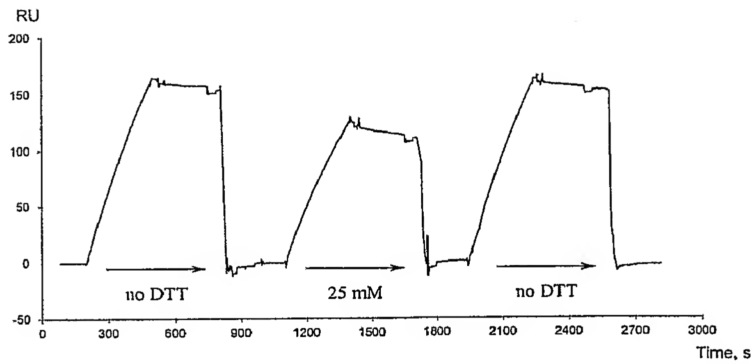
C



0.5mM stock solution of DGI-103 was incubated with indicated concentrations of DTT for 1hr at RT; then diluted 1:50 in Biacore running buffer (10uM DGI-103 final) and analyzed for binding with 2G12.

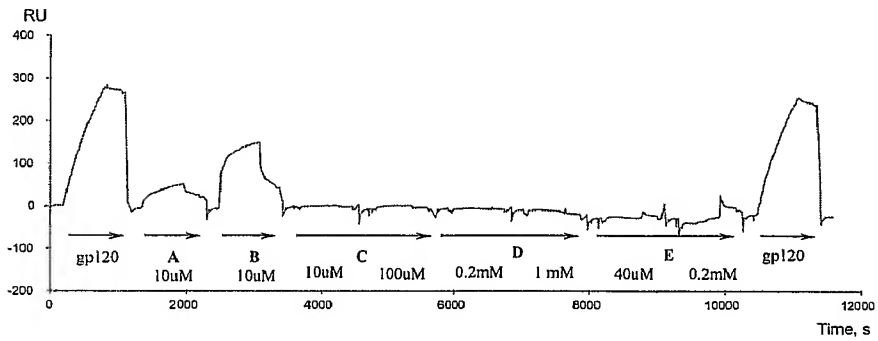
Figure 5

D



0.5mg/ml stock solution of gp120 (5uM) was incubated with indicated concentration of DTT for 1hr at RT; then diluted 1:50 in Biacore running buffer (0.1uM final) and analyzed for binding with 2G12.

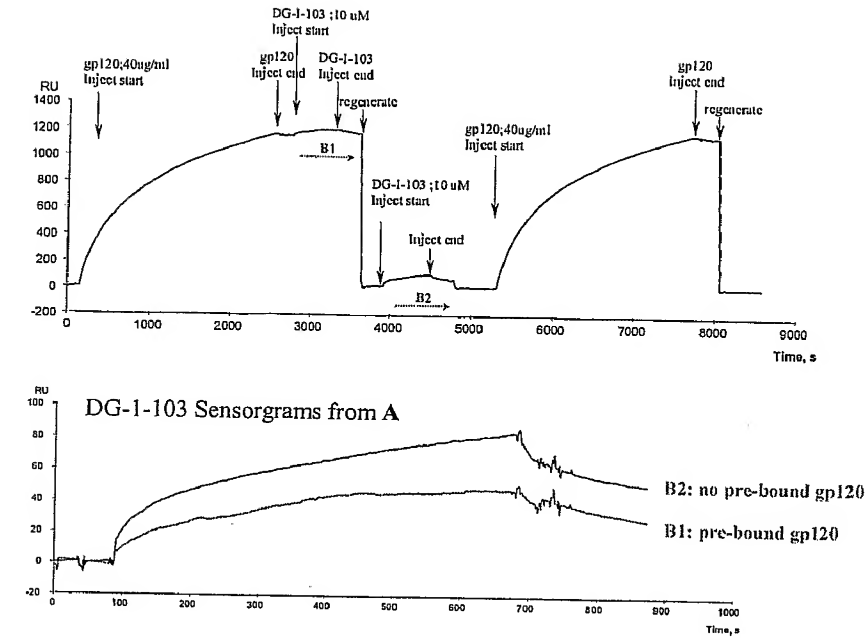
Figure 5



A – DGI-103 (high-mannose type glycan attached to the gp120$^{331-335}$ pentapeptide)-Cys is unblocked
B – DGI-152B (high-mannose type glycan attached to the gp120$^{316-335}$ 20mer-peptide)-Cys is unblocked
C – MMI-204 (hybrid-mannose type glycan attached to the gp120$^{331-335}$ pentapeptide)-Cys is blocked
D – DV-C53 (gp120$^{331-335}$ pentapeptide)- Cys is blocked
E – DGI-107 (high-mannose type glycan)

Figure 6

A


DG-I-103 ;10 uM
Inject start
gp120;40ug/ml
Inject start
gp120
Inject end
DG-I-103
Inject end
regenerate
B1
gp120;40ug/ml
Inject start
DG-I-103 ;10 uM
Inject start
Inject end
B2
gp120
Inject end
regenerate
RU
Time, s
DG-1-103 Sensorgrams from A
B2: no pre-bound gp120
B1: pre-bound gp120

B

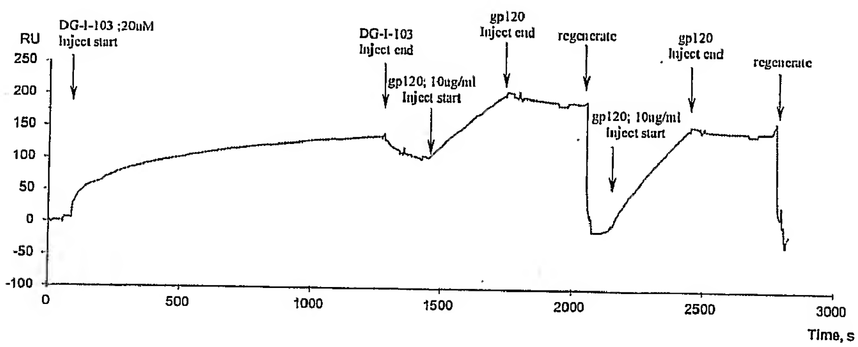

DG-I-103 ;20uM
Inject start
DG-I-103
Inject end
gp120; 10ug/ml
Inject start
gp120
Inject end
regenerate
gp120; 10ug/ml
Inject start
gp120
Inject end
regenerate
RU
Time, s

Figure 7